THE INFLUENCE OF ENVIRONMENT UPON BACILLUS PHYTOPHTHORUS AND THE POTATO BLACKLEG DISEASE

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INTRODUCTION

The blackleg disease of the Irish potato is one of the most widely distributed of the troublesome potato maladies. It has been reported from all parts of the earth, including all of the large, commercial potato growing districts. In Europe it is reported as ruining the main part of the potato crop in certain sections, being not general enough for an epiphytotic, yet severe enough to cause suffering in isolated commercial potato districts. In America the disease rarely reduces the crop more than 1%; in severest cases 75% has been reported.

REVIEW OF LITERATURE

For many years, a period extending roughly from 1875 to 1900, many descriptions of a potato disease similar to, if not identical with blackleg were published. In 1879 Reinke and Berthold (12) described a wet rot of the tuber which never contained a fungus. Since they were able to produce the disease in sound tubers by inoculation we have reason to believe that they were dealing with a disease resembling blackleg. In 1890 Prilleaux and Delacroix (2) described a disease of the stem which they thought was caused by "Bacillus caulivorus". So far as the organism is concerned there is reason to doubt the accuracy of the workers, but surely the symptoms of their stem disease answer very closely those of blackleg.

The connection between the wet rot of the tuber and the stem disease was not discovered until some time later. About 1897 Frank in Germany (Morse, 8 p. 81) showed that such a

connection existed. Frank's work was far more important than any that had been published up to that time. The name which was given to the disease was "Schwartzbeinigkeit" which has held in Germany and America to the present time.

Van Hall (3) in Holland in 1902 described a similar bacterial disease the causal organism of which he gave the name "Bacillus atrosepticus". From the report of Van Hall, it appears that his disease is very similar to Frank's "Schwarzbeinigkeit".

From 1902 to 1906 Appel (8,1) published papers on the blackleg disease and its causal organism. These are quite complete and he agrees that the organism named by Frank is the cause of the disease. He gave the organism the name, "Bacillus phytophthorus". Smith (15) in 1910 extended Appel's work and gave the most detailed descriptions of the disease and organism that had been published up to that time.

The disease was first found in the United States by Jones (5), in Vermont in 1906. The conclusion was that the organisms had come from Maine along with the seed which was used on the plot that showed the disease.

Harrison, (4) working in Canada, described a disease very similar to blackleg, but the causal organism seemed to differ slightly from Bacillus phytophtorus of Appel so he gave it a new name, Bacillus solanisaprus".

In 1901 Delaeroix described a bacterial disease of the potato stem to which he gave the name "brunissure". The bacteria which caused the disease were given the name

Bacillus solanicola. The disease described closely resembles typical blackleg, except for brown rather than black stem lesions.

Morse (9) reported the disease from Maine in 1907. The disease as reported by him was not severe, rarely exceeding one percent of the plants in the field.

In 1911, Pethybridge and Murphy (11) published a review of literature concerning the blackleg disease and the results of their investigations on a disease which occurred in Ireland and was similar to, yet somewhat different from blackleg. As there was a slight difference in the cultural characters Pethybridge and Murphy decided to give their organism a new name, Bacillus melanogenes.

Morse (8) in 1917 published a very extensive paper on blackleg, reviewing literature and comparing in a very detailed manner the organisms which he was able to obtain, He came to the conclusion that B. atrosepticus Van Hall, B. solanisaprus Harrison, B. melanogenes Pethybridge and Murphy, and three cultures obtained by himself should be considered as one species. There were slight cultural differences, to be sure, but they were so slight that, in his opinion, there is no reason to warrant several species.

The names that have been used in the past in connection with this disease of the stems and tubers are: black stem rot, black stalk rot, cellular rot, "Schwarzbeinigkeit", "brunissure", yellow blight and blackleg.

Character and Appearance of the Disease.

Blackleg is a disease of the stems and tubers of the potato plant, (Solanum tuberosum). The names that have been given to the

disease have, in large measure, been developed from the common appearance of it either on the stem or on the tubers.

As the name of the disease indicates, there is a blackening of the stem at the surface of the ground; in some cases it may extend higher. Before this black discoloration of the stem is visible other symptoms appear which alone are no definite criterion of the disease. Normal potato plants are of a spreading nature with a wide, branching top when the plant is of any considerable size. If the plant is attacked by blackleg this spreading habit is changed into a close growing top, bunched as it were, with the leaves more or less folded and curled along the midrib (5). Slightly later the leaves and stems begin to turn light green and later yellow and finally die.

If the plant is removed from the ground indentification of the disease is much more simple. Most likely the seed piece has decayed or is in the process of decay and on the sprouts leading up from the piece black streaks can be seen, which in most cases come to about the surface of the ground. In more severe attacks these black streaks or lesions on the stem extend one or more inches above the soil. In most severe cases these lesions have been known to extend to the lower leaves of the plant, causing a rapid death of the whole growth. This last type of development takes place only under the optimum weather conditions of moisture and temperature, namely, a high amount of moisture and a low temperature. Morse (8) reports no observations which show that the disease does not spread from one stem to another in the field

and that it does not progress downward from above ground lesions. Progress of the disease is always upward.

The bacteria grow on the underground parts of the plants and thus infect the small tubers as they are formed. This infection will cause a rot under conditions favorable for development of the disease. If these conditions do not prevail the bacteria lie dormant in the tubers and are taken from the field with them. In this way the inoculum for the succeeding year is insured and the organisms are present to cause tuber rot, should the right environmental conditions prevail.

Storage rot is not at all well known in America. In Ireland, where tubers are kept in storage pits, the rot becomes a very important aspect of the disease.

Blackleg is commonly an early season disease, being much more prominent in late June and early July than in August or September. The reason that the disease is more easily noticed early in the season is that the plants are small and hills that have been killed are thus more easily seen. Later the plants that are perfectly healthy grow and the gaps made by the skipped hills are filled in by their tops.

Soil conditions are very important in the development of blackleg. For optinim development of the disease there must be considerable moisture and low temperature. If the soil is moist, a low temperature necessarily follows, so where the one condition obtains, no doubt the other follows. The disease will be very severe if there are many heavy rains during the early part of the growing season.

Geographical Distribution

The blackleg disease is known in all of the large potato growing countries of the world. It has been reported from Germany, France, Belgium, Holland, England, Ireland, Canada and the United States. In this country the disease has been reported from all of the commercial growing districts. Morse suggests that the disease first entered Maine from the Province of New Brunswick, just across the Canadian border from one of the large potato growing areas of Maine. He also cites a case where diseased tubers were shipped in from Scotland to Colorado and then seed from that place sent to Maine, the disease appearing for the first time where the seed was used.

Economic Importance

There are two principal types of loss due to the blackleg disease, (1) tuber rot in storage, and (2) loss of plants and tubers in the field. Of the first loss, that of tuber rot, very little is known in America. It is in Ireland and other countries in which potatoes are stored in pits that this loss is keenly felt. Pethybridge and Murphy (9) report that sometimes whole storage pits are invaded by the black leg organism and the entire contents utterly destroyed. In America the tubers are usually kept in cold storage where potatoes are grown on a large scale and in cool, dry cellars when on a small scale. For best storage results the temperature should be kept as low as possible without freezing the tubers, and there should be as little moisture as

possible. Under these conditions the bacteria remain dormant, thus doing no harm.

In America, however, there is more or less loss of the plants in the field. In some isolated and rare instances losses as great as 75% of the standing plants have been reported, but according to Morse (8) these instances are only interesting and not of practical value in determining the economic loss due to the disease. In Maine he reports that in many instances there are but a few scattered plants in a field which are diseased. He also says that a loss of 1 or 2% is not uncommon, and a loss of 5% is considered severe by the growers. Pethybridge and Murphy (11) report that in Ireland losses often approximate the whole crop when seed badly infected with the organism is used.

In the United States in the year 1918 the government estimate of the loss due to blackleg was about 2,000,000 dollars.

On the whole, then, it might be said that the disease is not very destructive at any one time but that it takes its toll of the potato crop each year, aggregating in any one year or series of years a tremendous loss to the potato industry of this country.

Rosenbaum and Ramsey (14) have found that the organism which causes the disease lives over in the tuber and not in the soil or in old tubers which overwinter in the soil. Tubers which show no visible signs of rot are, no doubt, placed in storage along with the sound tubers and kept from rotting by storage conditions.

In this way diseased tubers may overwinter and be used for seed the following spring, as they show no signs of any disease. It is in this way, Morse (15) says, that the disease is propagated from year to year.

Spread of the disease in the field has never been reported, and Morse (8) says that such spread is very improbable, for the bacteria which cause the disease are very susceptible to drying and thus cannot stand the desiccation which must naturally take place if they are to be spread about in the field.

Etiology of the Causal Organism

The name most commonly given to the organism which causes blackleg is Bacillus phytophthorus Appel. It is a small rod, measuring about .5 by 1.4 microns. It is motile, with one to five peritrychic flagella. The organisms occur singly or in pairs. There is no formation of spores and involution forms are seldom found. In one instance Harrison (4) reports that he found involution forms of his organism when grown at 37° C.

Control

According to Jones & Vaughan (7) and the Board of Agriculture and Fisheries of London (18), there are four methods of control which are of some value, namely, careful selection of seed, treatment of seed with some good disinfectant, rogueing, and drainage of the land if wet or the use of only such land as is naturally dry.

Each of these methods of control gives some measure of disease reduction but not to exceed thirty or forty per cent under the best conditions. Seed selection and seed treatment combined control the disease almost perfectly. If the cultural suggestion is followed there is no reason why control cannot be complete. Rogueing is resorted to in order that there may be no chance for infected seed to be used or even allowed to be mixed with healthy tubers in storage.

Morse (10) carried on extensive field experiments, proving conclusively the relative values of seed selection, seed treatment and a combination of the two in control of blackleg. He found that the disease was reduced about one-third by either seed selection or seed treatment, and that by a combination of the two methods almost complete control was obtained.

There is considerable resistance shown to the disease by the different varieties of potatoes. Morse (8) states that the Irish Cobbler is very susceptible, while the late variety, Green Mountain, is highly resistant to blackleg. Jones(6) reports that in Europe there are several varieties in each country visited by him, which show resistance to blackleg. Appel, as reported by Jones, states that "While no varieties have been shown to be entirely free from the disease, there is evidence that thick skinned, starch rich, late varieties are in general more resistant than thin skinned starch poor, early varieties".

Control of this disease is so simple and so perfect that there is no reason for any considerable occurrence of it anywhere at any time.

Temperature Relations

The blackleg disease, so far as can be learned from the available literature, has never been studied in relation to environment. Field observations have been made and a few field experiments have been carried on, but little accurate experimental data has been published.

Morse (8) of Maine and Pethybridge and Murphy (11) of Ireland have made the latest contributions to blackleg literature. However, they have not been interested in environmental relationship except in so far as it is very evident by field observations.

There is very close correlation between moisture and temperature and the development of the disease in question. Blackleg of potatoes is found (only?) during (excessively?) wet seasons with low temperature. As low, poorly drained places give much the same condition, the disease may be found during a rather dry season. Farther than this we have no knowledge as to the effect of environment on the development of the disease.

Harrison (4) states that "the optimum temperature (for growth) is from 25°- 28° C., good growth occurs at 20° , but at 37° C., growth is very scanty, and as has been previously stated, involution forms develop ".

Morse (8) worked out the temperature for the optimum development of the causal organism, Bacillus phytophthorus Appel. He says, "the optimum temperature for the six pathogens studied is not far from 25° C. although no attempt was made to determine this within a variation of 5° C. above or below." Van Hall

reported that vigorous growth occured at 27° C., although he did not attempt to determine the temperature for the greatest growth.

In his experiments, Morse did not attempt to grow the organism at a temperature lower than 5° C. At this temperature he observed growth after 84 to 96 hours; at the optimum temperature visible growth was noticed in 11 hours. Smith (15) in his description of B. phytophthorus states that the organism will not grow at 1° C. or any temperature lower. He found that freezing a culture in bouillon caused the death of 90% of the bacteria. In the field it has been found by Rosenbaum and Ramsey that the organisms that cause blackleg do not overwinter in the soil or in tubers left in the soil. This was determined in Aroostook County, Maine, and at Norfolk, Virginia, during seasons that were shown to be average by comparison with weather reports of previous years.

Smith (15) gives the maximum temperature at which growth may take place as 36° C. Morse found that no growth took place above 34°C., in liquid medium, and none above 32 or 32.5°C. on agar slants. He believes that " undoubtedly the somewhat lower maximum temperature for growth on agar was due to the rapid drying out of the surface of the medium". He checked his results several times to make sure he was correct, as Harrison had reported the maximum temperature growth for his organism, Bacillus solanisaprus, as 37°C.

EXPERIMENTAL PROCEDURE

The workers on Bacillus phytophthorus or the blackleg disease have determined, within a rather wide range, the temperature for optimum growth, not the relation of moisture or temperature to the development of the disease. The nearest approach to the optimum temperature for bacterial growth was worked out by Morse (18) and found to be 25° C., but the optimum was not determined within a range of five degrees above or below. This temperature gives no accurate clue as to optimum temperature, it simply shows that there is a stronger and more rapid growth at 25° than at 20 or 30° C. The optimum could be either 28° or 21° so far as Morse's work is concerned.

The object of the present experiment was to determine within a range of 3 or 4° the optimum temperature for growth. In the determination of this it would also be possible to determine the effect of a wide range of varying temperatures upon the bacteria. The main purpose of this more or less accurate determination was to make possible a comparison between the bacterial development as influenced by temperature and that of the disease. It was planned to grow potato plants at varying temperatures and thus determine the optimum, minimum, and maximum temperature for the development of the disease. This phase had to be abandoned, however, due to complications met with in determination of the optimum temperature for growth of the organism.

Technique

Nowhere in literature including bacteriological literature, was anything to be found concerning the more or less accurate determination of bacterial growth in culture media, aside from the plating method. This method, considering the number of readings desired, was physically impossible. It was thought that some means could be devised such that the work would be reduced as much as possible and at the same time give an approximately accurate determination of the amount of growth.

The American Board of Health (16) devised a method for determination of the turbidity of water, using standard solutions of silicon dioxide (SiO_2) as a means of compariosn. Acting upon this as a suggestion, a set of standard suspensions of SiO_2 was made up in test tubes of uniform size so as to have no error in reading due to varying thickness of the solution. A culture of B. phytophthorus was grown in nutrient broth and compared with the suspensions as prepared. The standard set and the media proved very unsatisfactory. The suspension did not in the least approximate the turbidity of the bacterial growth, nor was the colored nutrient broth easily comparable to a clear suspension of the silicon dioxide. Another more satisfactory substance was needed if a good standard was to be had.

Since barium sulphate is almost insoluble in water and very finely divided, it was decided to try it. A microscopic examination was made of the silicon dioxide and the barium

sulphate and it was found that the barium sulphate was much more finely divided, with the exception of a few large particles that could not be separated by agitation. A set of standards was made up in uniform test tubes, and a comparison was made with a culture of the bacteria in nutrient broth. The suspension itself was very similar to the turbidity of the bacterial growth and thus one of the weak points of the silicon dioxide suspension was overcome.

Next, it was necessary to change the culture medium to one that was the same color as the set of standards. Morse states that Bacillus phytophthorus grows exceedingly well in Uschinsky's solution, so that solution was given a trial. It proved to be ideal, as the color was the same as water and it furnished good medium for the growth of the organism.

It might be said that B. phytophthorus develops in Uschinsky's solution with no surface growth and with a finely granular or flocculent precipitate. Due to this fact it is very easy to get uniform turbidity thruout the culture with slight agitation. It was at first thought that the sediment would interfere with accurate comparison of the growths, but later it was decided that the amount formed was probably determined by the extent of bacterial development. No doubt there is some ground for doubting the accuracy of this statement, yet it seems such would be within the probable experimental and human error.

Making of Uschinsky's Solution

Because Bacillus phytophthorus grows extremely well in Uschinsky's solution it was necessary to make up standards, the heaviest of which were very turbid. It was found that a suspension of .8 of a gram of barium sulphate suspended in 500 c.c. of distilled water gave a turbity slightly heavier than the best bacterial growth. This was used as a stock solution and dilutions were made from it.

There were small lumps of the barium sulphate which could not be made to go into suspension by agitation, but as these seemed to be constant and unavoidable, they were disregarded. The entire amount of water and barium sulphate solution placed in a tube was 10 c.c. The tubes were of uniform diameter so that the same volume of suspension was observed in making all readings.

The solutions were made up with the following dilutions, the figure on the left indicating the amount of stock barium sulphate suspension, and the figure on the right the amount of distilled water used to each 10 c.c. of standard:

1	2	3	4
1/2-9 1/2	1-9	1 1/2 - 8 1/2	2-8
5	6	7	8
3-7	4-6	5-5	6-4

Culture Media

As mentioned before, Uschinsky's solution seemed to be ideal for the carrying out of the experiment. It was made according to the following formula, a slight diversion from

some in common use:

Formula for Uschinsky's Solution:

Ammonium lactate	7 gms.
Asparagin	4 gms.
Calcium chloride	1 gm.
Dipotassic phosphate	2.5 gms.
Glycerin	40 c.c.
Magnesium sulphate	.4 gm.
Sodium chloride	7 gms.
Distilled water	1000 c.c.

The medium was sterilized by heating in streaming steam 15 minutes for four consecutive days. After this, the hydrogen-ion concentration was taken and found to be $P_h = 6.8$, a truly neutral solution.

Method of Experiments

Ten c.c. of Uschinsky's solution was placed in each test tube. All tubes had previously been selected to insure unifrom diameter. These were sterilized as stated above, fifteen minutes in streaming steam four consecutive days.

Inoculum

The culture of B. phytophthorus used was a strain isolated by Schultz in this laboratory several years ago. As it has been carried in artificial culture for a long period, it seemed advisable to test its pathogenicity and its cultural number according to the chart of the Society of American Bacteriologists.

Two sound potato tubers were used for the pathogenicity determination. They were carefully washed and then placed in corrosive sublimate solution, 1-1000 dilution, for 5 minutes. The tubers were then rinsed with distilled water several times to remove any of the corrosive sublimate which remained on the

One tuber was inoculated with a young agar slant culture obtained from the stock culture. The other was treated in the same manner with the ommission of the organism.

In four days the inocculated tuber showed distinct indications of rot, while the check remained sound and normal. Isolations were made from the rotted tuber and streak cultures thus obtained.

One of these streak cultures was used to inoculate a new slant, a sound tuber, and a set of tubes containing the media called for in the chart of the Society of American Bacteriologists. In this way the pathogenicity and the cultural number could be determined and a transfer kept, from one original culture.

The tubers which was inoculated rotted completely in about ten days. The culture media inoculated gave results which, when recorded by the descriptive chart, gave the following number: 221.1113522. Morse working with three cultures of B. phytophthorus isolated by himself and with a culture each of B. solanisaprus, B. melanogenes, and B. atrosepticus, found that the cultural number for all of these organisms was identical with the one in question.

From these, then, it could very accurately be said that according to previous descriptions the culture of Bacillus phytophthorus tested was virulent and pure.

Inoculation of Tubes

In carrying out a bacteriological experiment it is necessary to use a uniform, duplicable inoculum. In older

cultures this would be of minor importance perhaps, but in recording growths in young cultures it might be a very great factor in determining the turbidity of bacterial growth.

To start with, a tube of Uschinsky's solution was inoculated with a small bit of culture from an agar slant. This tube was incubated at 25°C. for 48 hours. At this time a second tube of Uschinsky's solution was inoculated with one loopful of this culture. This second tube was treated as the first had been and from it a third tube was inoculated and incubated in the same way. In repeating this so many times it was thought that any by-products might be done away with and a fairly duplicate culture obtained.

From the third culture, tubes of Uschinsky's solution were inoculated with a standard 2 mm. wire loop. As soon as they were made, these cultures were placed in incubators with temperatures varying from about 3° to 36°C., with an interval of approximately three degrees.

Experimental Results

Culture turbidity readings were taken every 24 hours for 8 days. With a little practice, comparisons could be read to a great degree of accuracy. Where it was possible, readings were made between the standard suspensions, such readings being marked plus or minus the nearest suspension.

The results of these readings are given in Table I. Both the range and the average temperature are given. Rather wide ranges are noted in some cases. These, however, are not so great a factor as the data would lead one to believe. Where the

TABLE I Showing Growth Determined by Comparative Method (Series I)

Culture Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Temperature Range	3.0-5.0	5.8-7.0	8.0-9.8	10.4-13.0	12.2-14.4	16.0-18.1	18.0-21.2	22.6-32.2	23.8-24.8	28.2-28.7	29.2-30.3	31.0-33.0	32.4-34.5	33.8-42.0
Average Temperature	3.0	6.25	8.12	11.52	13.71	17.01	19.55	25.45	24.05	28.41	29.94	32.36	33.18	37.37
Age in Days														
1	0	0	0	0	0	0	0+	1	1	1	0+	0+	0	0
2	0	0	0	0	0	0+	0+	1	1	1	0+	0+	0	0
3	0	0	0	0+	0+	1	1	2	3	1	0+	0+	0	0
4	0	0	0+	0+	1	2	2	3	3	1	0+	0+	0	0
5	0	0+	0	1	2	2	3	4	4	2	0+	0+	0	0
6	0	0+	1	2	2	3	4	4	5	3	2	0+	0	0
7	0	0+	1	2	3	4	5	4	5	5	3	0+	0	0
8	0	0+	2	3	4	5	5	4	5	6	5	0+	0	0

difference is greater than 2^{o} , it was for a very brief period, except in the case of No. 8. There, the temperature made a sudden rise on the sixth day, from about 22^{o} to 30^{o}, enough to retard greatly or even stop the growth in those cultures.

In culture No. 8 there was a reduction of growth at the time this increase of temperature took place. Instead of the expected increase on the seventh day, it remained the same as for the sixth day. For the first six days the temperature for No. 8 did not vary more than 2^{o} , between 22.6 and 24.6^{o} C. Aside from this one irregularity, the temperatures followed rather closely the averages for the given periods.

There was a gradual increase in growth at the lower temperatures as time passed. At first, no growth took place below 17^{o} C. Each succeeding day, for several days after this was noticed, growth was found at a lower temperature, until 3^{o} C. was reached. In about twelve days growth appeared even at that temperature, but to a limited extent, not increasing much in the time before the cultures were discarded.

At the temperatures above the optimum growth appeared, as in the lower temperatures, with the lapse of time. In this case, however, the decline in growth was much more rapid than at the lower temperatures. In other words, the decline from the optimum growth is much more rapid on the higher temperature side than on the lower.

Figures 1 to 6 show graphically the growth on the second thru the sixth, and the fourteenth days. The first five

figures show quite regular curves, with an optimum at about 25° , at least 23 and 28°C. Because of the relative inaccuracy of the comparative methods, some curves seem to show that a very good, if not an equally abundant growth of the organism took place at temperatures slightly above or below the optimum.

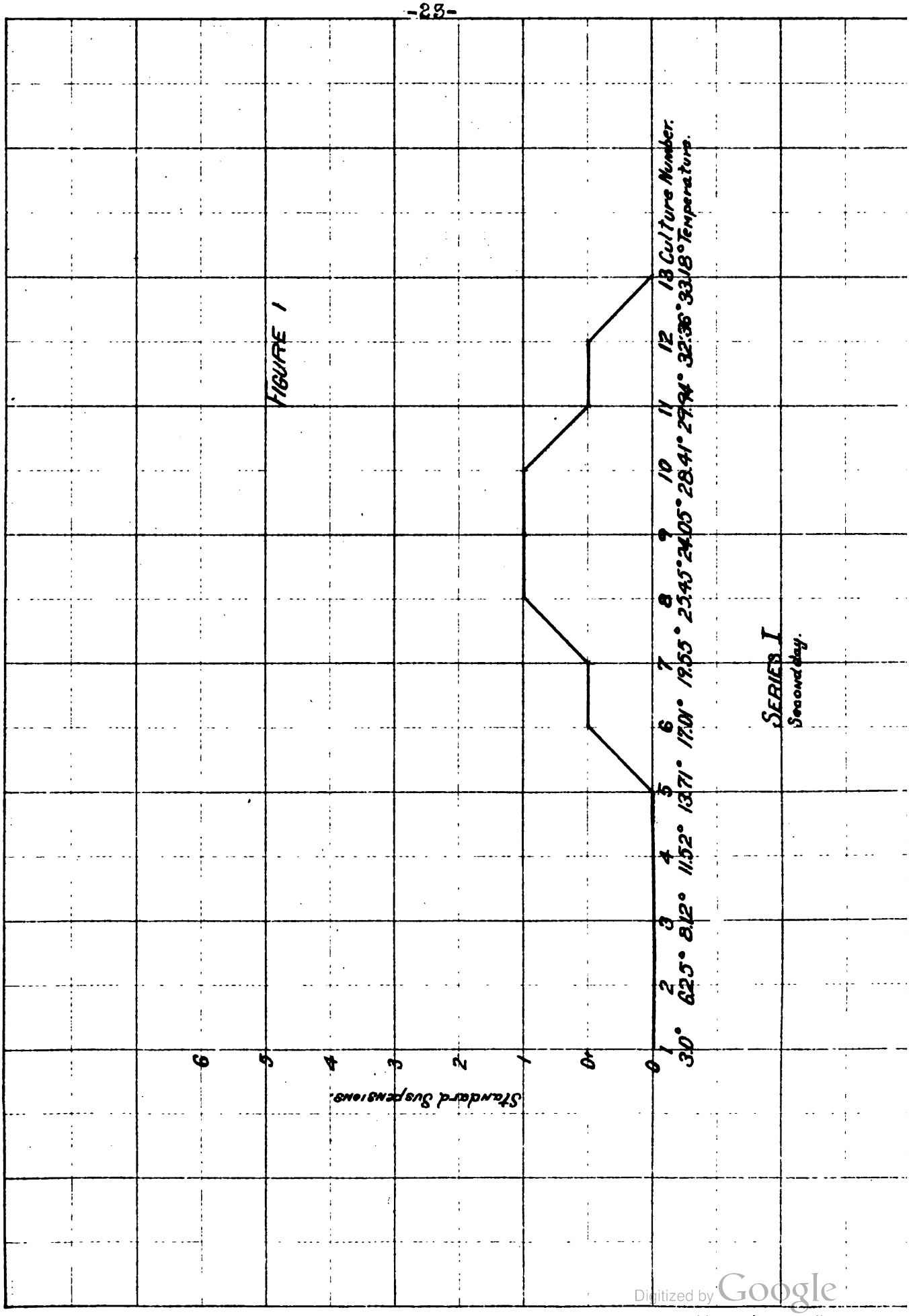
Readings were made on the fourteenth day, just before the cultures were discarded. Figure 6 gives the graph representing the growth up to this day. The growth, as indicated by cloudiness, started at 3°C. and rose evenly to 17° . The growth seemed about equally abundant at 17.01°, 19.55° and 25.45°, the last temperature being actually about 23°. At 24.05° there was less growth, and still less at 28.41° . At 29.94° the cloudiness was much greater, exceeding to a considerable degree that of the acknowledged optimum of 25° . The amount of growth then dropped off rapidly at 32.56° to none at 33.14° .

While this peculiar bimodal growth could not be taken very seriously, it caused considerable thought, as it closely paralleled bimodal curves which have been found in plants such as cabbage and tobacco when diseased and grown in the greenhouse at varying temperatures.

To make sure that the bimodalism was not the result of conditions peculiar to this first trial, it was decided to repeat the experiment to see whether under conditions, as nearly similar as could be obtained, the bimodal growth would appear.

FIGURES I TO 6 SHOWING GROWTH CURVES
FOR THE SECOND, THIRD, FOURTH,
FIFTH, SIXTH AND FOURTEENTH
DAYS, RESPECTIVELY. (SERIES I)

FIGURE 1



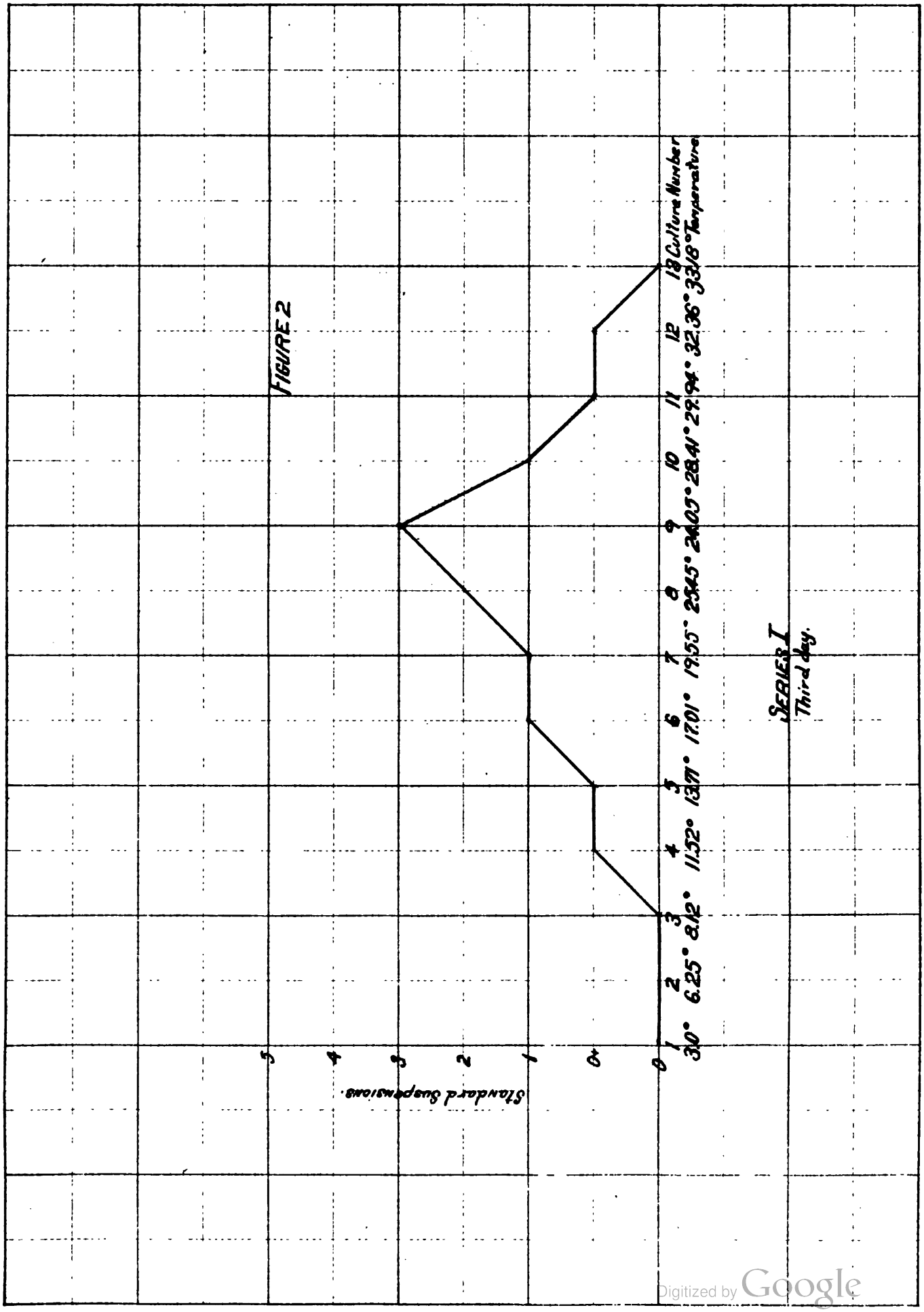
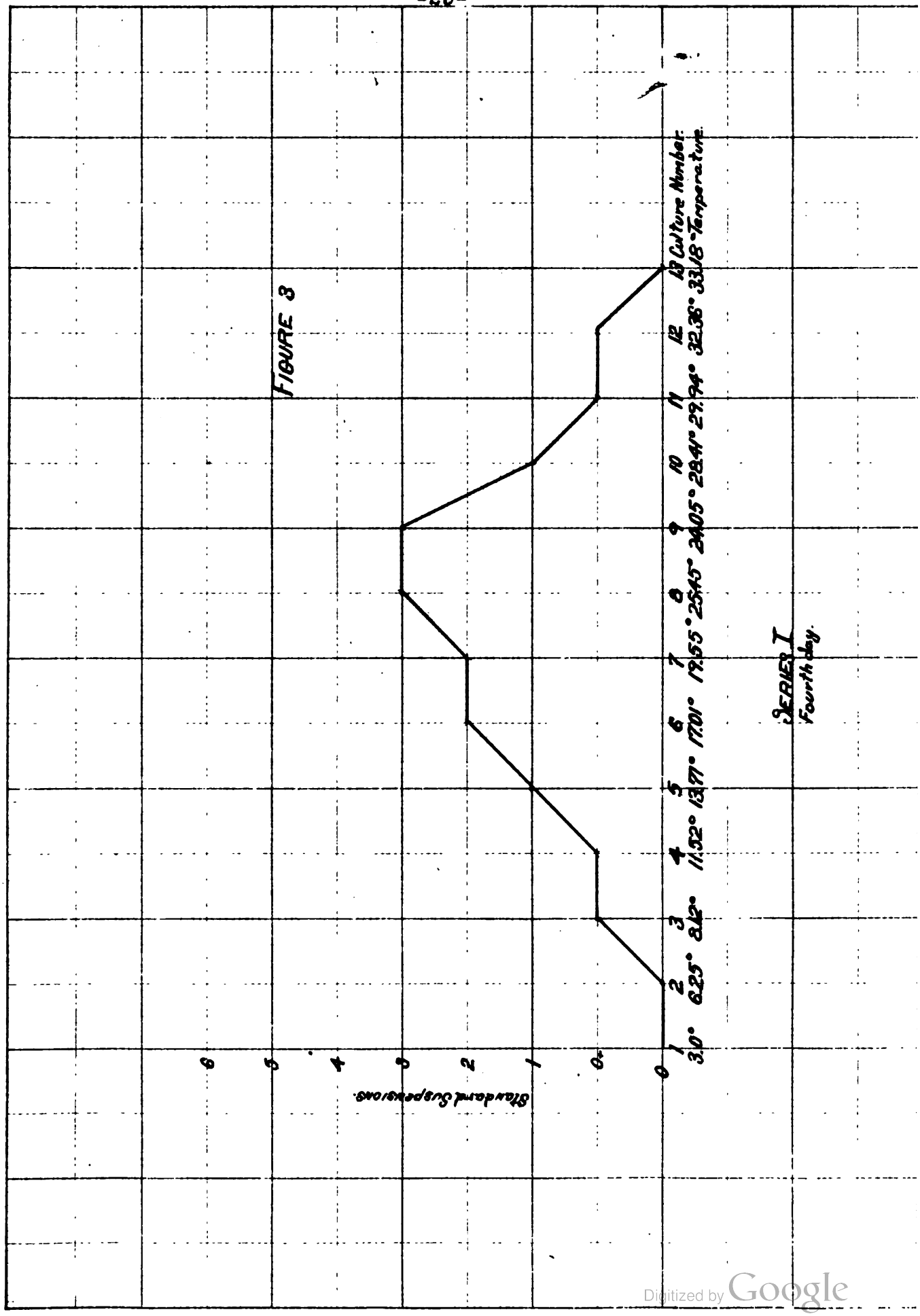
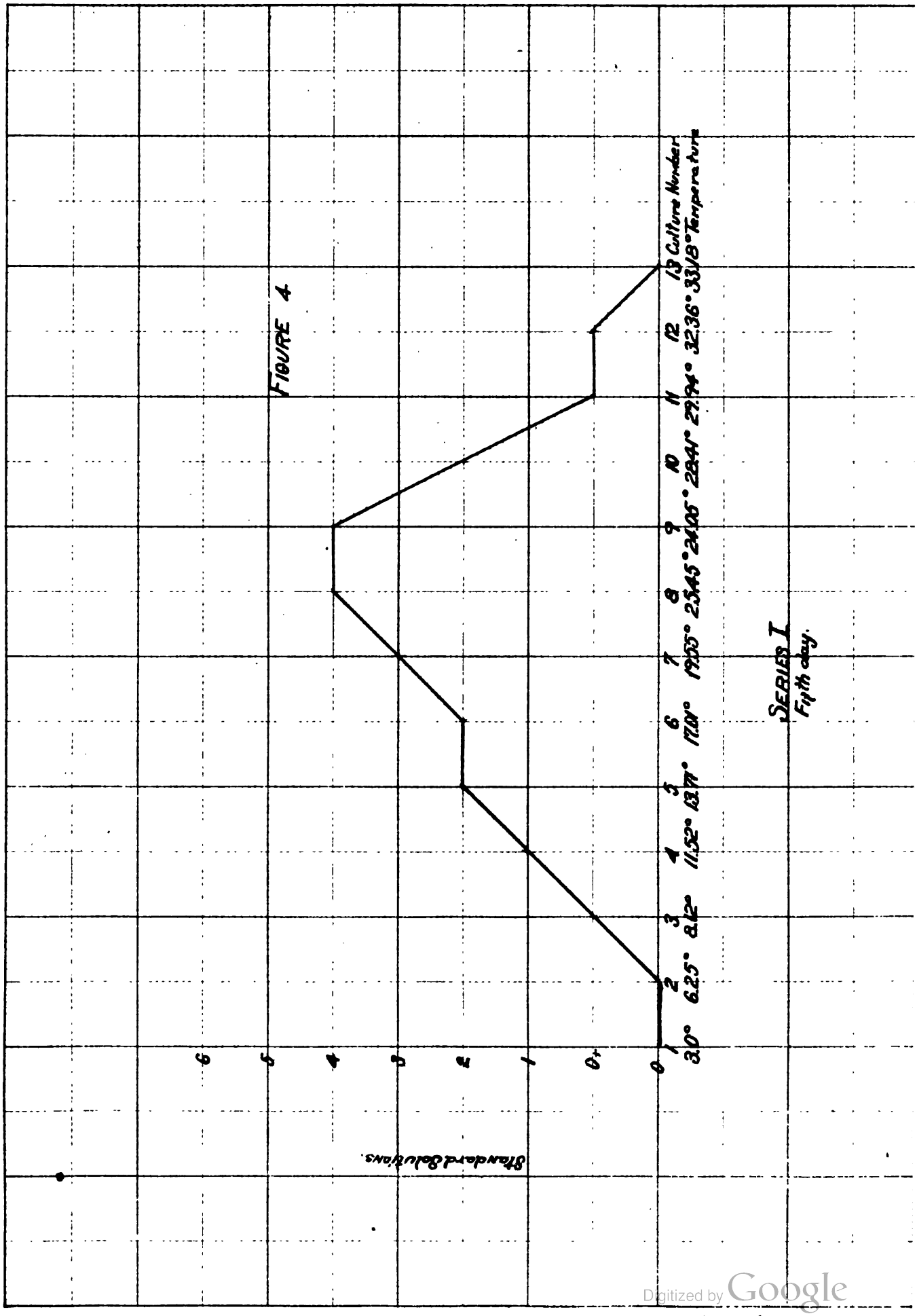


FIGURE 2





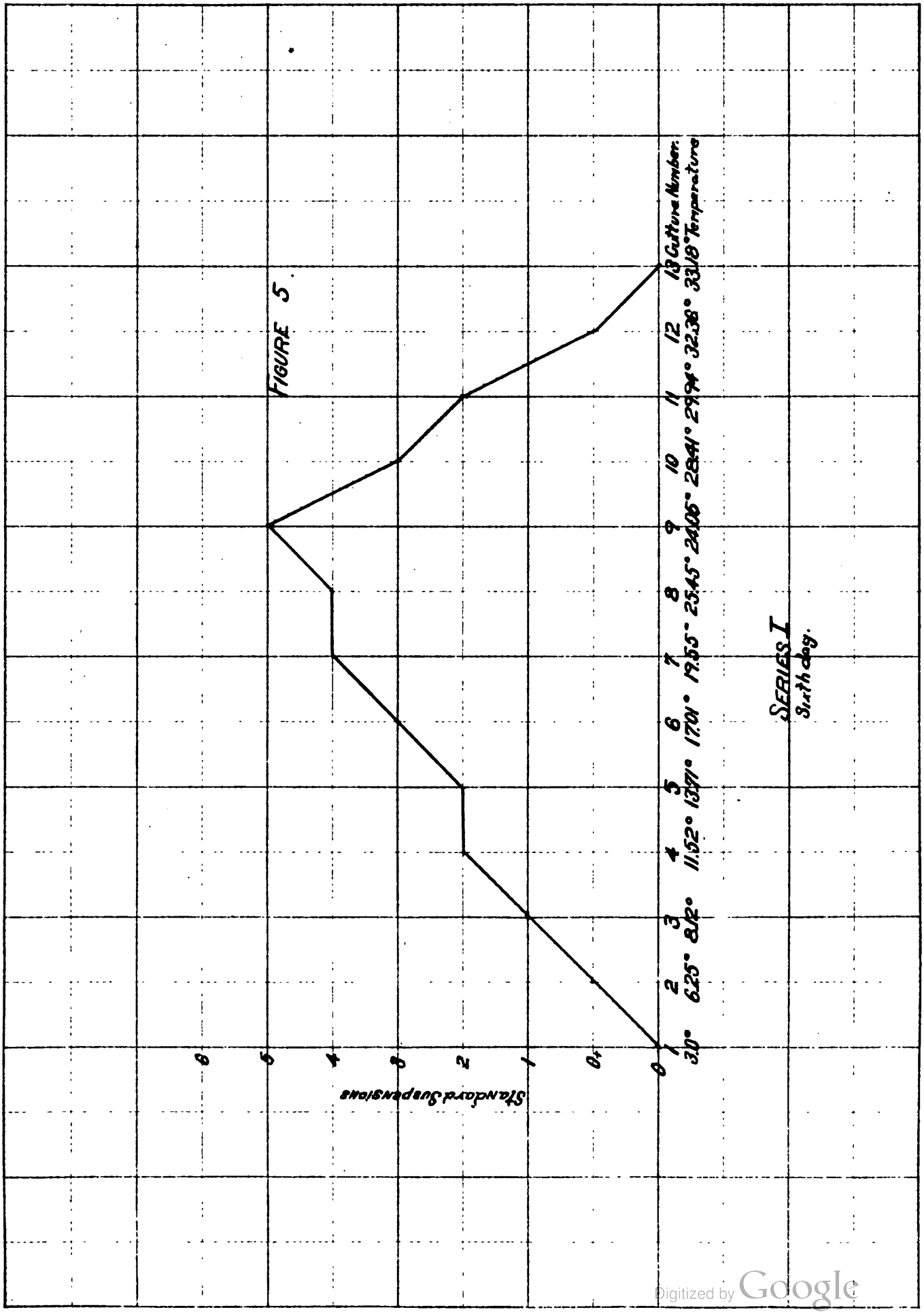
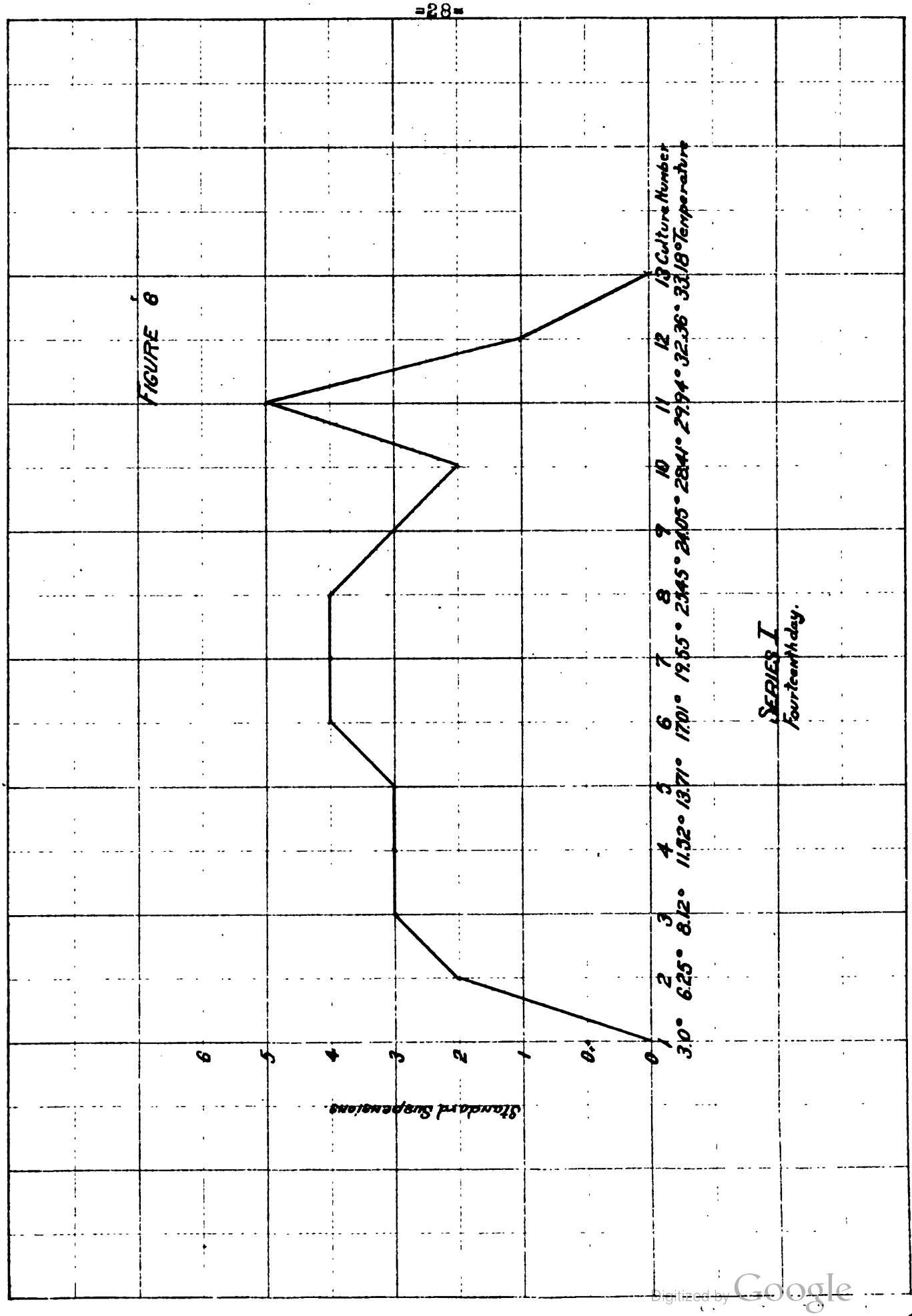


FIGURE 5.

FIGURE 8



EXPERIMENTAL PROCEDURE- SERIES II

Duplicating as accurately as possible the first series, a second was started at the temperatures used before. Comparisons were made with a new set of standard suspensions of barium sulphate as often as was thought necessary.

Using the new set of standards and plotting curves from the data obtained, the curves are slightly distorted. In making up the suspensions the first five tubes contained amounts of stock barium sulphate suspension in quantities differing by $\frac{1}{2}$ c.c. Because the heavier suspensions were made up in strengths varying by 1 c.c., the amount of growth is made to look greater in the first five numbers than it actually is. The general shape of the curve, however, is not changed.

In figures 7 to 12 are given graphically the results of the readings of growth. As the second set of standard suspensions was made up with larger units than the first, small variations in cloudiness are not so closely recorded.

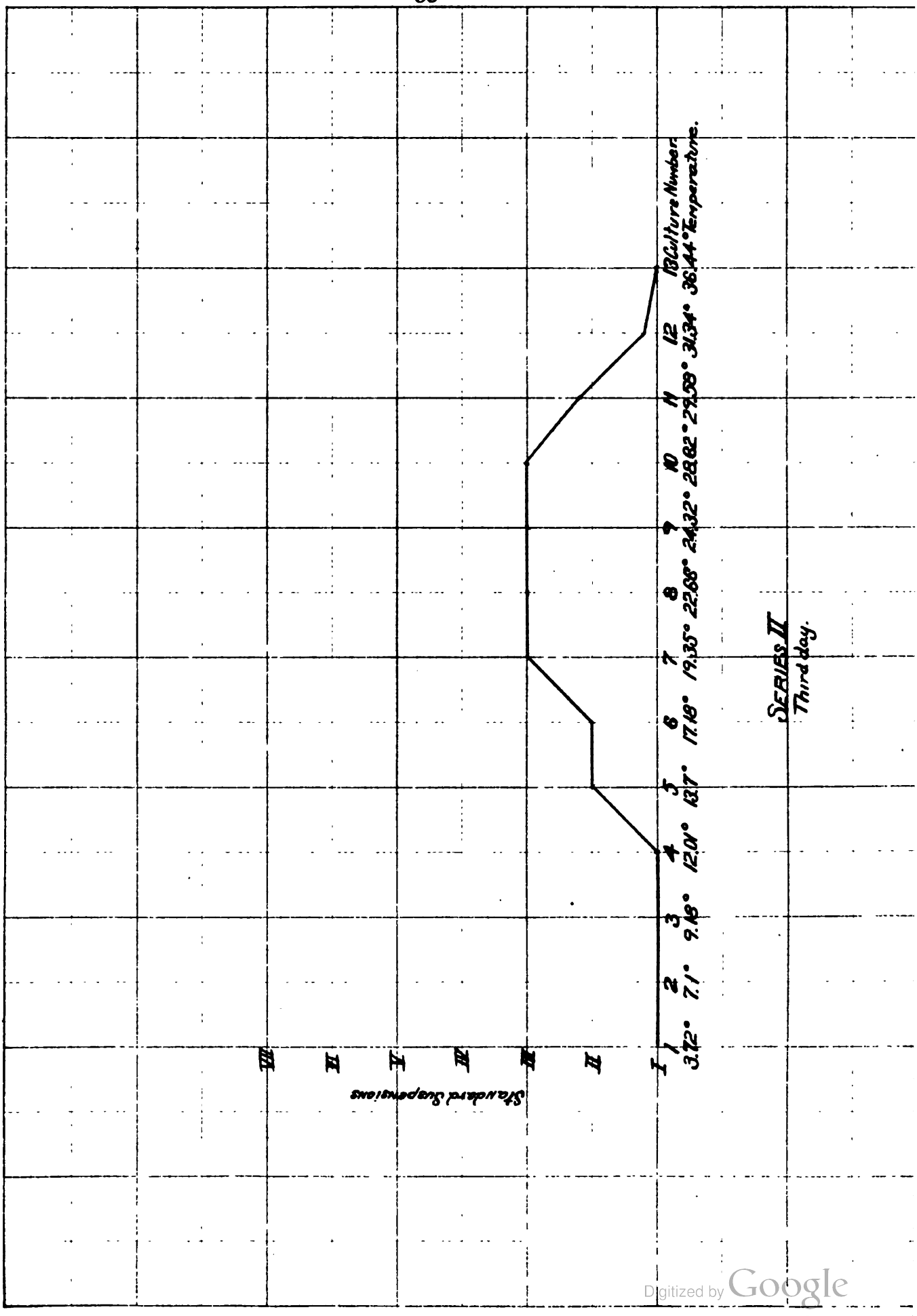
In the readings of the third, sixth, and eighth days no definite optimum was apparent. Equal amounts of growth seem to have taken place thru a rather wide range of temperature. The curves also show no bimodal growth on the days in question. Figures 10, 11 and 12, readings for the tenth, eleventh and twelfth days, respectively, show distinctly the bimodal growth noticed in the first series on the fourteenth day.

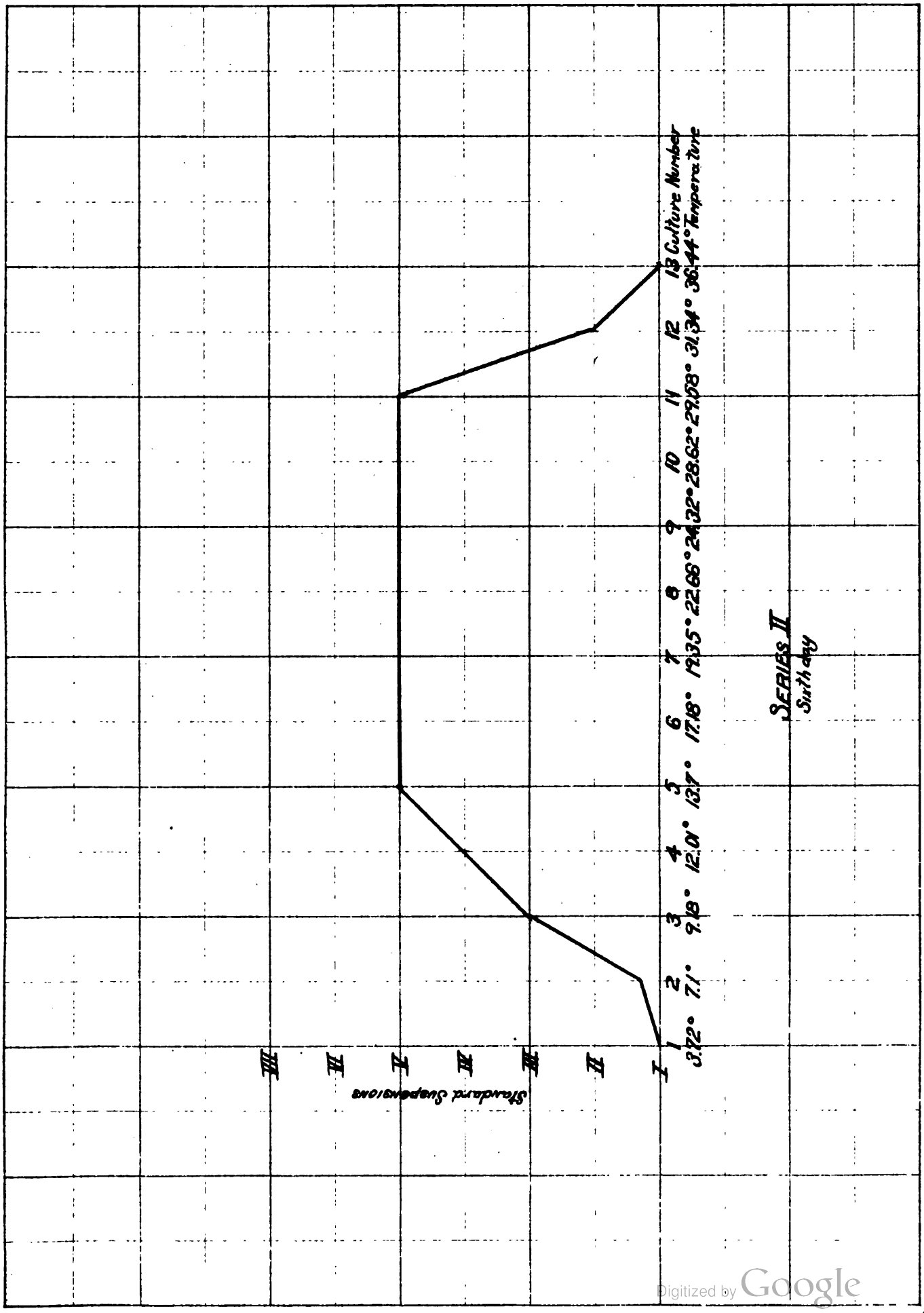
From the repetition of this peculiar growth, it was the more firmly believed that a bimodal development was constant, with approximate optimums at 25° and 30° C. As the comparative method of growth determination is rather crude, it was decided to resort to a more accurate method of determination and thus prove, if possible, the assumptions drawn from the first two series.

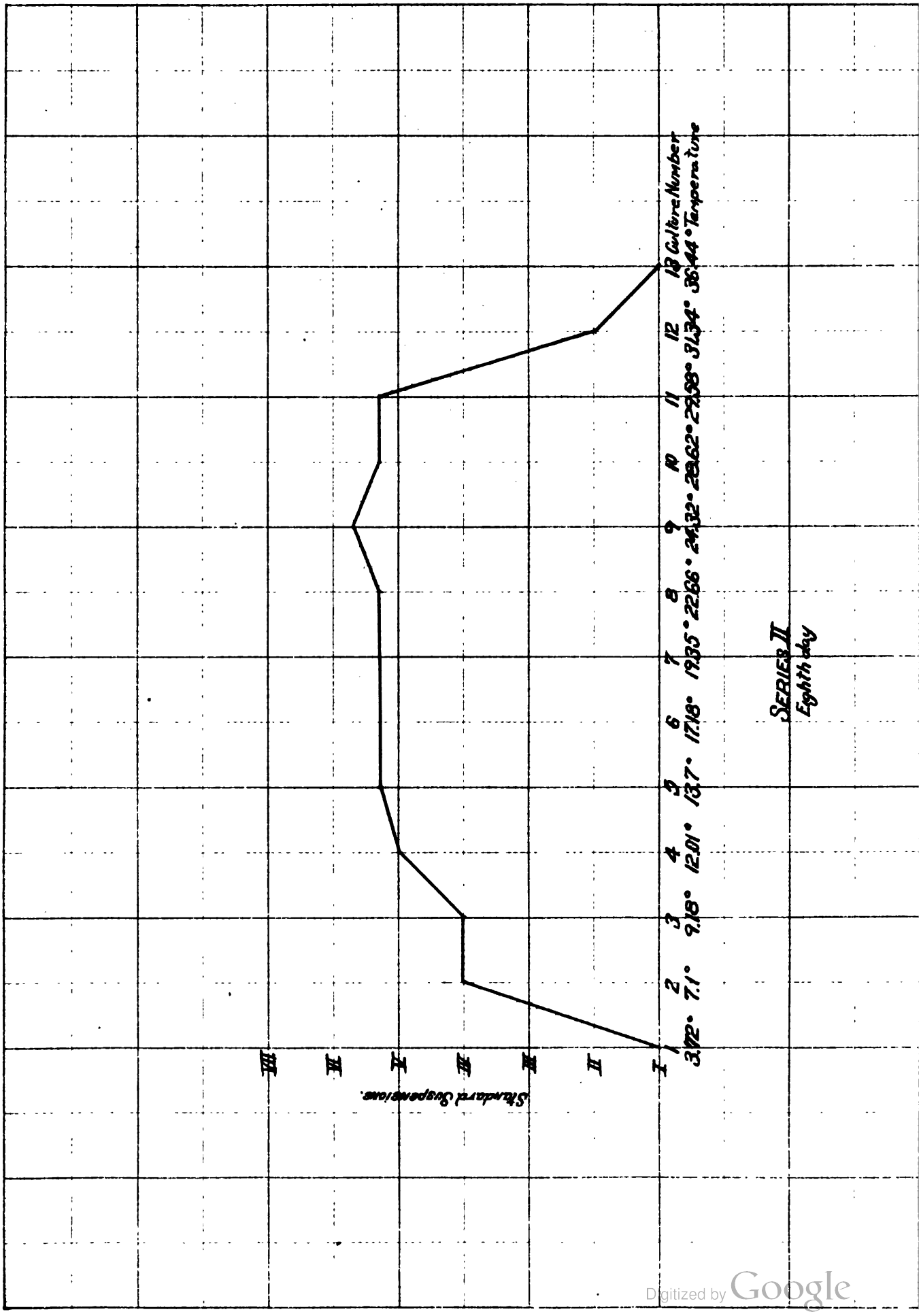
TABLE II Showing Growth Determined by Comparative Method (Series II)

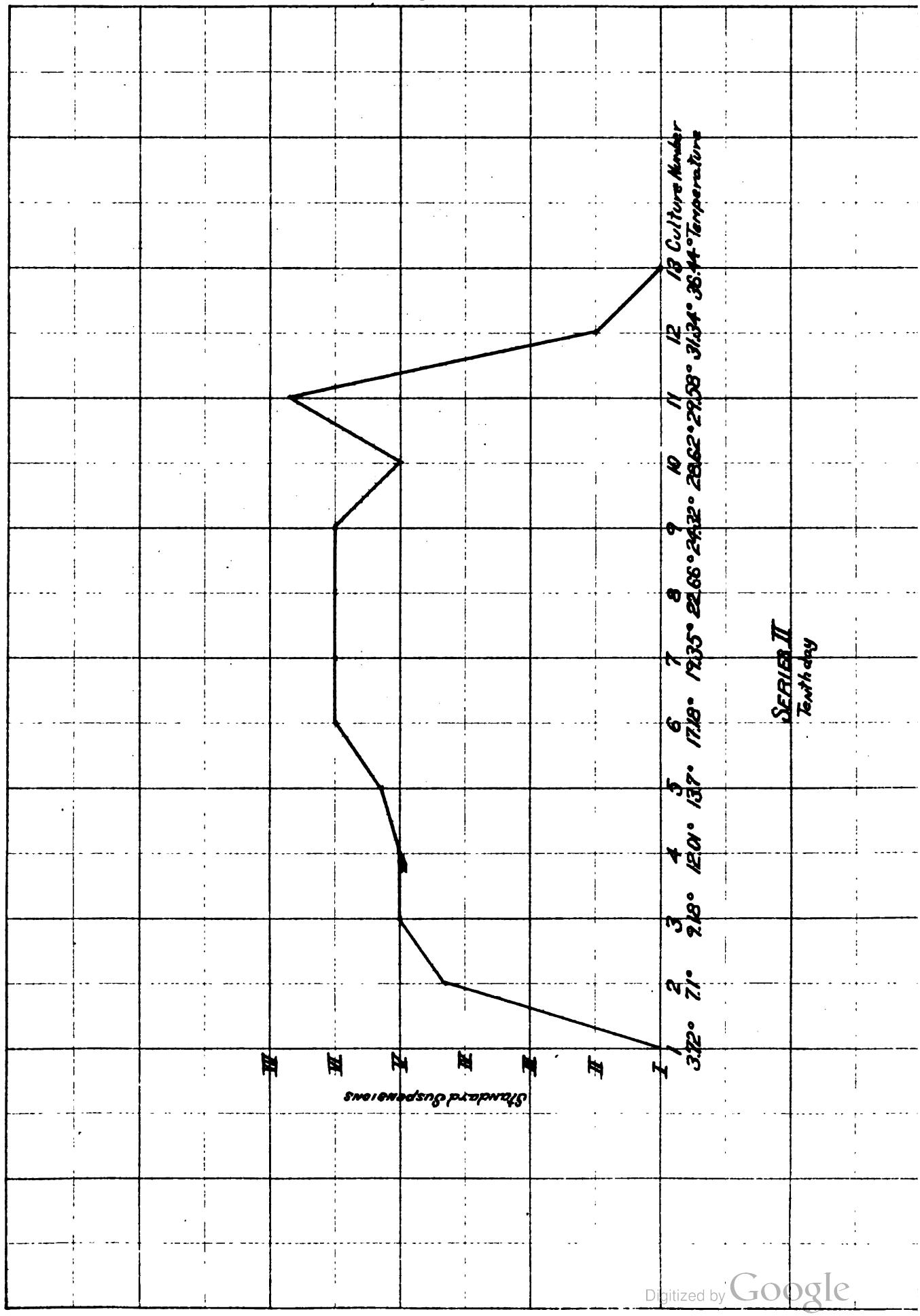
Culture Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Temperature Range	3.0-4.5	6.0-8.0	7.2-10.2	11.0-13.4	12.6-15.1	15.8-19.0	18.4-21.8	21.5-25.0	22.0-25.4	28.0-29.0	29.0-30.0	29.0-32.5	34.0-44.0
Average Temperature	3.72	7.1	9.18	12.01	13.7	17.18	19.35	22.66	24.32	28.62	29.58	31.24	36.44
Age in Days													
1	I	I	I	I	I	I	I	I	I	+I	I	I	I
3	I	I	I	I	II	II	III	III	III	III	II+	I+	I
6	I	I+	III	IV	IV	V	V	V	V	V	V	II	I
8	I	IV	IV	V	V+	V+	V+	V+	VI-	V+	V+	II	I
10	I	IV+	V	V	V+	VI	VI	VI	VI	V	VII-	II	I
11	I	V	V	V	V+	VI	VI	VI-	VI	V	VII-	III	I
12	I	V	V+	V+	VI-	VI	VII-	VI	VI	V	VII	III	I
14	I	V	V+	V+	VI-	VI	VII	VI	VI	V	V	III	I
16	II	V	V+	V	VI-	VI	VII+	V+	VI	V	V	III	I

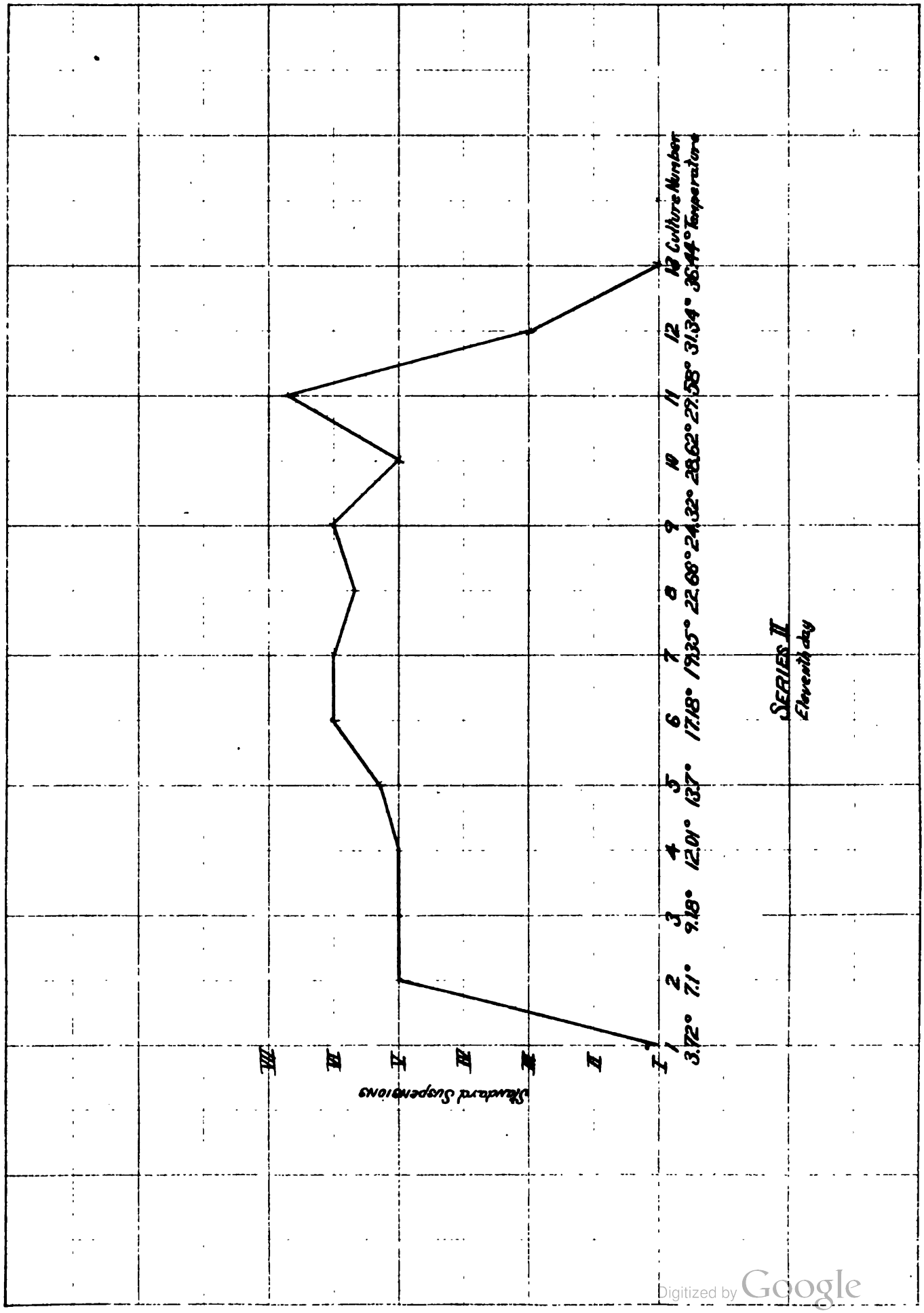
FIGURES 7 to 12, GROWTH CURVES
FOR THE THIRD, SIXTH, EIGHTH, TENTH,
ELEVENTH, AND TWELFTH DAYS, RESPECTIVELY.
(SERIES II).

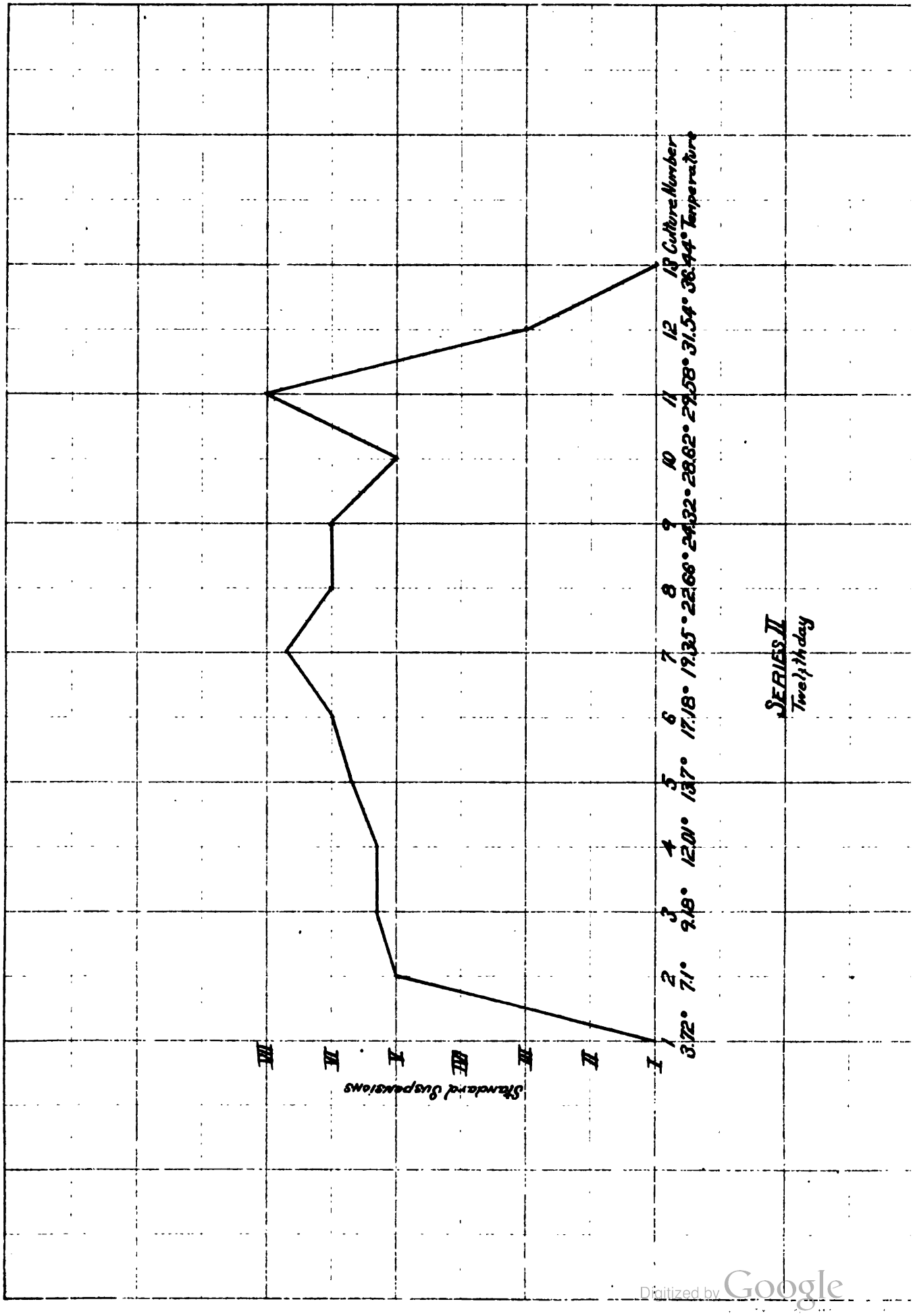












DETERMINATION OF OPTIMUM TEMPERATURE BY PLATING

Determination of bacterial growth by gross comparative methods is, at best, merely a relative procedure. Because of the danger of inaccuracy entailed in such a method it was thought wise to make plate determinations to obtain more accurate results. As this method gives comparative results it serves its purpose, for growth is inherently a comparative thing.

Technique

Uschinsky's solution made according to the formula given previously was used, 15 c.c. being placed in each test tube. Three test tubes were placed at each temperature so as to take no chances with contaminations or poor inoculations.

After two, four and six days incubation at the various temperatures, dilution plates were made in duplicate. Nutrient peptone agar was used in plating. The dilutions made were 1 to 90,000. The plates were incubated at 25° for 48 hours, at which time colony counts were made.

Results

The first set of plates, made at 48 hours, were especially good, with but few contaminations and the two plates for each temperature corresponding closely. In the set of plates made at the end of four days there were many contaminations, so that an accurate count was impossible. These were not considered at all because of this fact. The plates made at the end of six days were good, containing few contaminations and the two sets corresponding

quite closely. In the series at this time cultures at 30 and 32 degrees were lacking due to an accident.

The results of the series just described are given in Table III and graphically shown in figures 12, 14, 15, and 16.

The great variation in range of temperature does not show accurately the influence which it exerted on the growth of the organism. The higher temperatures were for a very short period of time, not more than two or three hours at most, but as the temperatures were recorded once in twelve hours it would lead one to believe that the deviation lasted for that period. The average temperatures approximate closely those which have been determined in the same incubators for a rather long period.

The growth of this series exhibits a close parallel to those recorded at a greater age by the comparison with standard suspensions. The maximum growth took place at 25° , with a secondary maximum at 30° , and a noticeable falling off at 28° C. The growth as recorded by colony development shows a corresponding amount of growth so far as it goes and speculation alone can determine the behavior at 30 and 32.5° .

TABLE III , Showing Growth Determined by Plate Method(Series III)

Culture Number	4		5		6		7		8		9		10		11		12	
Temperature Range	11.8°-16.40°		13.2°-17.2°		16.2°-19.0°		18.8°-21.5°		22.2°-25.3°		25.0°-26.2°		27.0°-28.6°		29.0°-31.0°		30.0°-34.0°	
Average Temperature	13.00°		14.64°		17.84°		20.65°		23.98°		25.53°		28.11°		29.88°		32.45°	
Age in Days 2	4	3	0	2	3	35	4	9	81	102	280	305	29	11	108	90	1	2
4	91	83	0	3	500	415	525	575	734	488	488	423	367	427	507	465	241	246
6	271	246	313	351	185	230	277	354	635	671	1107	1176	486	396				

FIGURES 13 - 16 GROWTH CURVES

FOR THE SECOND AND SIXTH DAYS (SERIES III).

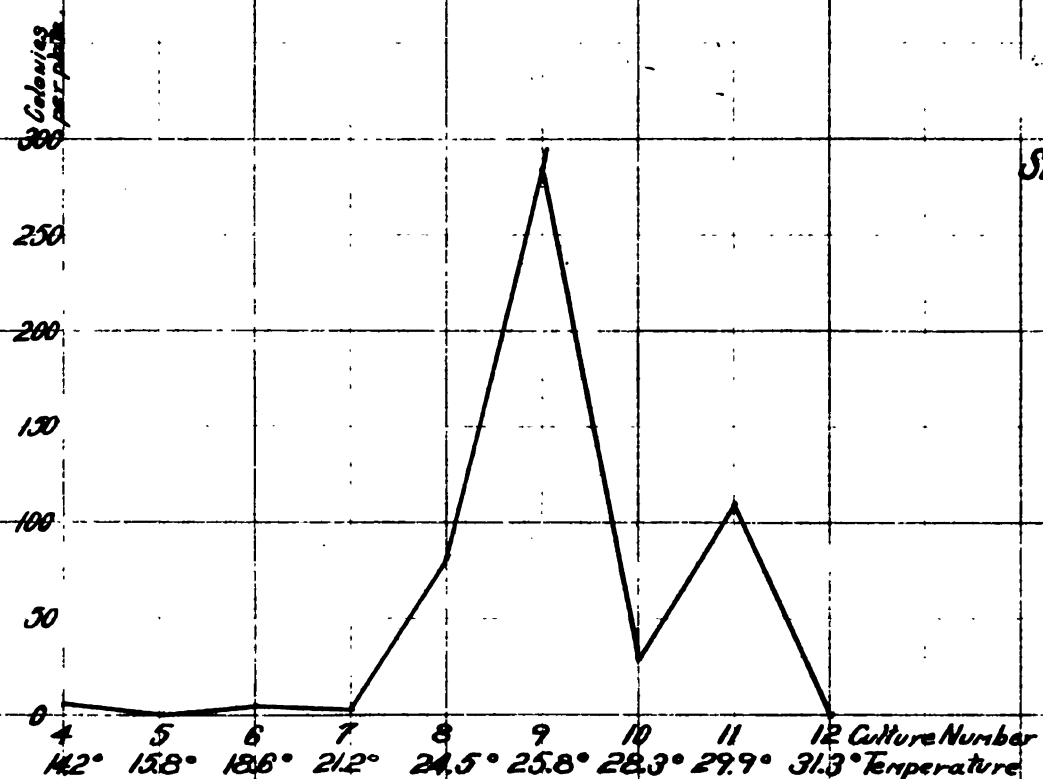


FIGURE 13

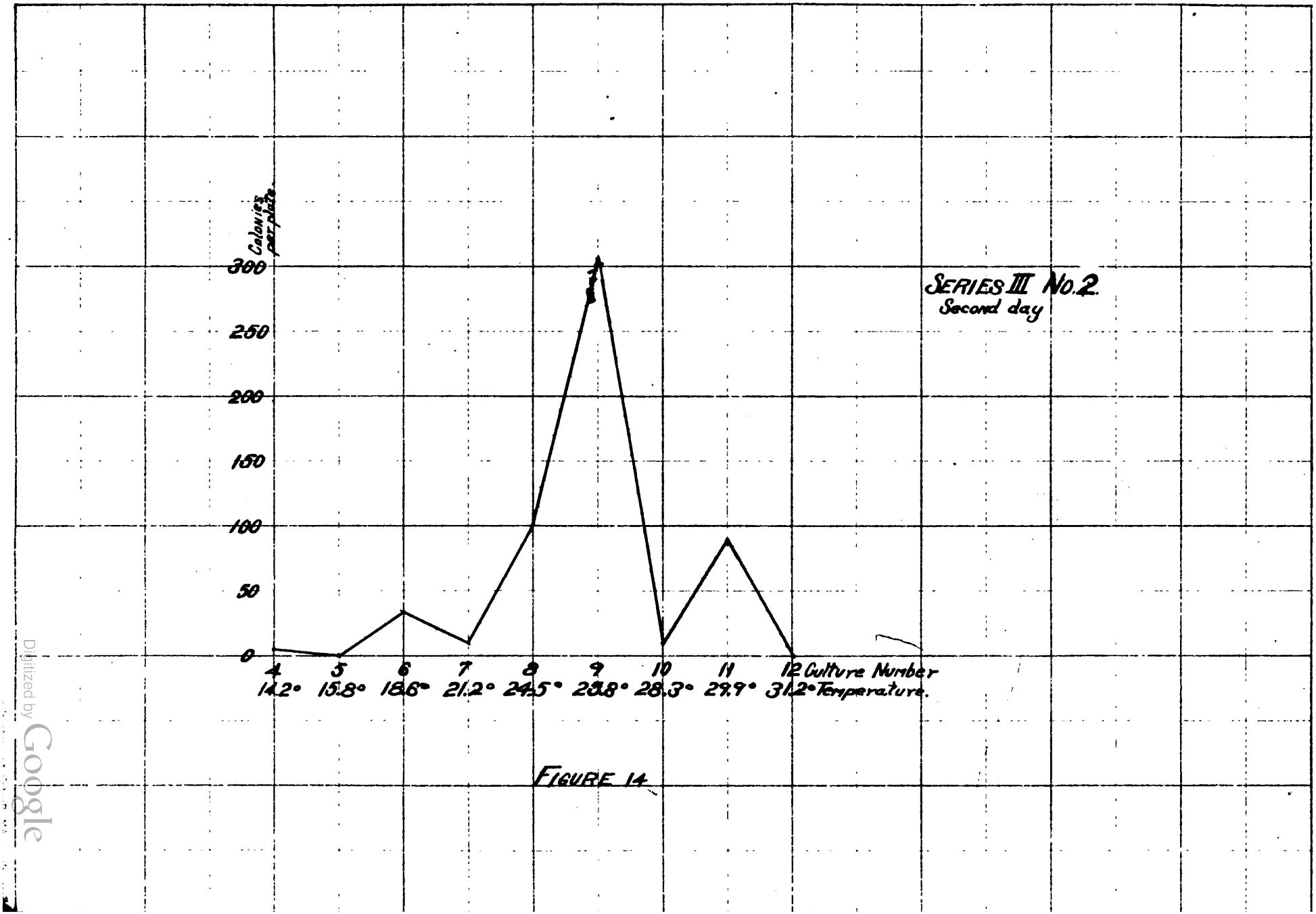


FIGURE 14

SERIES III No 2
Sixth day

Colonies per plate

1150
900
750
600
450
300
150
0

4 5 6 7 8 9 10 11 12 Culture Number
130° 14.64° 17.84° 20.85° 23.98° 25.63° 28.11° 29.89° 32.45° Temperature

FIGURE 15

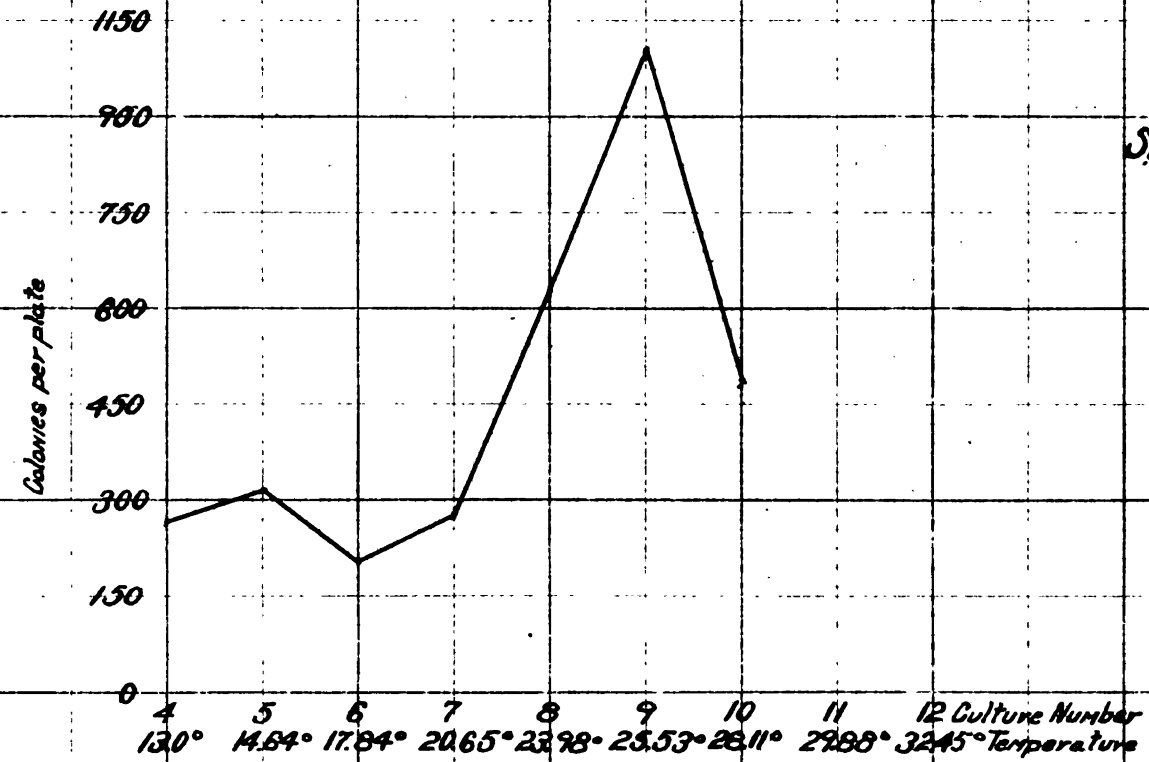


FIGURE 16

Conclusion as to Optimum Temperature

Series I, determining growth by comparison with standard suspensions of barium sulphate for six days, points rather clearly to 25° as the optimum temperature for growth.

Series II, using the same method of growth determination but with less fine gradations of standard suspensions does not distinctly point to any one temperature as an optimum.

In Series III, where plating was resorted to, a primary optimum was found at 25° C., with a secondary optimum at 30°. The secondary optimum did not give nearly so much growth as did the primary, yet it was sufficient to be called an optimum.

From the three series described above, it is quite possible to give 25° C. as the primary optimum temperature and 30° C. as the secondary optimum temperature for the development of Baccillus phytophthorus.

DETERMINATION OF GENERATION TIME

Neither the determination of bacterial growth by comparison with standard suspension nor by plating methods gives very accurate results. In the comparative method there are several factors which enter in and could be the source of considerable error. In the first place no inoculation, however accurate - it may be, can be the same thruout any considerable series of tubes if made with a wire loop or even with an accurately calibrated pipette. Secondly , there is opportunity for considerable human error in making the comparisons with the standard suspensions. For these two reasons and for others of minor importance, this method gives at best only a crude approximation of bacterial growth.

By plating methods, at the end of any given time more accurate determination of growth is possible. In this method the human error and the error due to long and varying temperatures are done away with. Still the variation in the amount of inoculum may cause considerable error here, as with the comparative method.

The bimodal growth found in the cultures grown at several different temperatures and determined by these first two mentioned methods seemed important enough to warrant accurate determination of rate of division of the bacterial cells, or the generation time. Since bimodal growth was found to take place between 20 and 32° C., it was decided to duplicate growth at these temperatures only.

First Series

Technique:- Uschinsky's synthetic medium was used for determination of the generation time, as it had already been used in the previous series. Likewise the medium was made up according to the formula given before. The P_H value was determined and found to be 6.6. In the first two series it was found to be 6.8, which is not fundamentally different.

Inoculum:- A twenty-four hour old culture in Uschinsky's solution was used as inoculum. This culture was carefully obtained by the method described under the first two series.

Inoculation:- Fifty c.c. of Uschinsky's solution was placed in a small flask and inoculated with .5 c.c. of the above mentioned twenty-four hour old culture.

Plating of Cultures:- After inoculation the cultures were well shaken and dilution made of 0.5 c.c. of the inoculated medium in 150 c.c. of sterile distilled water, or a dilution of one to three hundred. One cubic centimeter of this water suspension was placed in each of four Petri dishes and plated in nutrient peptone agar. The plates were then placed in a twenty-five degree incubator and allowed to grow for three days, after which the bacterial count was made.

The inoculated flasks of Uschinsky's medium were placed in incubators with temperatures as follows:-

8	9	10	11	12
20.8°C.	25.3°C.	28.0°C.	30.0°C.	32.5°C.

The temperatures which were read six times during the twenty four hours, did not vary more than four tenths of one degree, and then only in Nos. eight and twelve.

After the required time for growth the cultures were removed from the incubators and dilution plates made as in the beginning.

Results:- The plates which were made from Uschinsky's solution immediately after inoculation were placed in a 25 degree incubator and left there for three days. At the end of this time colony counts were made so as to determine the number of bacterial cells used per c.c. in the inoculation of the flask.

These plates were made in quadruplicate and the results obtained were as follows:-

	1	2	3	4
At 22°	52	85	114	25
At 25°	40	1	3	3
At 28°	1	23	21	111
At 30°	75	75	100	110
At 32°	120	107	27	125

The determinations do not check very well, yet they give an indication as to the amount of inoculum used. It will be noticed that the culture at 25 deg. had apparently the smallest amount of inoculum.

After twenty four hours of incubation the cultures were again plated out and placed in a 25 degree incubator for three days, as was done with the first plates. At the end of this time

they were examined. It was found that the dilutions made were not nearly great enough and that the colonies were so thick that even an estimation of them with a Frost plate counter was impossible.

One interesting fact was noticed which has some bearing on the experimental evidence of a bimodal development of B. phytophthorus. When placed in a series on a black desk in the order in which they were grown, that is, to the left the plate from 22°, next that from 25° , etc., it was distinctly noticed that there was a much greater colony development at 25 and 30° than at 22, 28 or 32° . In order that the writer's judgment might be confirmed several other people were asked to locate the plates showing the greatest colony development. In all cases it was found to be at 25 and 30° . This is interesting simply because it shows once more that even under more carefully controlled conditions a bimodal growth took place, when approximately the same amount of inoculum was used in each case. At 25° it was found that there was a smaller amount of inoculum than in any of the others, and yet the greatest colony development took place in the plates made from that culture. This is one more proof, then, that the optimum temperature for the growth for B. phytophthorus is near 25° C. It also gives another indication of the bimodal growth of the organism in Uschinsky's solution, under the conditions of the experiment.

Second Series

Technique:- The same method of procedure was followed in this series as in the first series under "generation time". The medium used was the same, being made up at the same time.

Inoculum:- A thirty hour old culture of Bacillus phytophthorus obtained as in the first series, was used to make the inoculations.

Inoculation:- Fifty cubic centimeters of Uschinsky's solution in a flask was inoculated with 0.5 c.c. of the above mentioned culture.

Plating of Cultures:- Immediately after inoculation the cultures were well shaken, and dilution plates made as follows: 0.5 c.c. of the inoculated medium was placed in a water blank containing 150 c.c. making actually a dilution of 1-300. One c.c. of this suspension was placed in each of four Petri dishes and plated with nutrient peptone agar. Previous to inoculation the flasks of medium were placed in their respective incubators that they might be at the desired temperature or nearly so when inoculated. Immediately after inoculation the flasks were returned to the incubators and allowed to incubate for twenty three hours.

The temperature readings were taken frequently during the time of incubation. The averages for that time are as follows:-

8	9	10	11	12
23.5°	25.8°	28.5°	30.0°	32.4°

After twenty-three hours of incubation the cultures were removed and dilution plates made in the following manner: 0.5 c.c. of the culture was placed in a 500 c.c. water blank. After care-

shaking one c.c. of this suspension was placed in each of four Petri dishes and plated with nutrient peptone agar. In this case the actual dilution was 1-1000. These plates were placed in a 25° incubator and allowed to incubate for three days, at which time a colony count was made.

Results:- At the end of three days of growth the plates made at the time of inoculation were counted. Since the dilution is known this count gives the basis for learning the actual amount of inoculum used in the cultures per c.c. This is the first step necessary in the determination of generation time.

The colony count is recorded in the following table:

	1	2	3	4
At 23.5°	23	33	36	35
25.8°	10	12	10	9
28.5°	29	29	30	32
30.0°	14	22	21	12
32.4°	10	4	6	4

The plates made at the end of incubation contained so many colonies that a direct bacterial count was impossible. On account of this it was decided to estimate the number with the aid of a Frost plate counter. As comparative results are quite as valuable as absolute ones it was thought that this method would not detract greatly from the value of the data.

To test the accuracy of the method of estimating plates, it was thought wise to check it with an actual count. The

colony count of one of the plates was estimated and then the actual count made. It was found that the error in this case was slightly more than 3%, which in itself is not great, even for rather accurate work.

The estimated colony count for the plates made at the end of the incubation period was as follows:-

	1	2	3	4
At 23.5°	504	820	750	850
25.8°	Discarded	2590	2660	2450
28.5°	3800	3780	3500	discarded
30.0°	2800	2870	2900	2880
32.4°	840	920	800	910

Determination of approximate generation time:- In order to determine the generation time, or the time for the division of one cell, it is necessary to know the cells present to begin with and the number at the end of any given period of incubation.

As four plates were made for each temperature, it was deemed best to average the three plates with the nearest counts and use that number as the actual count. If the four were near together they were all used in this determination.

The average plate counts are shown in the following table:-

	At Inoculation	After Incubation
At 23.5°	34.6	806.6
25.8°	10.1	2533.3
28.5°	30.3	3360.0
30.0°	19.0	2860.0
32.4°	15	867.5

The following table gives the actual bacterial counts per c.c. at the different temperatures at the time of inoculation, and after incubation:-

	At Inoculation	After Incubation
At 23.5°	10.380	806,600
25.8°	3,030	2,533,300
28.5°	9,090	3,360,000
30.0°	5,700	2,860,000
32.4°	4,500	867,500

The determination of the number of cell divisions during any given period is necessary for determination of the generation time. By substituting in the following formula the values given in the preceeding table, it is possible to get the number of times any cell divides in the period taken, namely, 23 hours:

$$N = \frac{\text{Log } (B/A)}{\text{Log } 2}$$

N = number of generations or the number of divisions of one cell in 23 hours.

A = the bacterial count per c.c. at the time of inoculation or the initial count.

B = the bacterial count after incubation or the final count.

After the number of cell divisions during a given period is known, it is necessary to determine the actual time taken for one cell division. The following formula is used in this determination:

$$G.\ T. = \frac{T}{N}$$

G. T. = generation time.

T = time in minutes of incubation period.

N = number of generations as determined above.

Results:- By following the scheme above, the generation time at the temperatures used was determined. The results are as follows:-

At 23.5° - 220 minutes

25.8° - 142.2 minutes

28.5° - 162.3 minutes

30.0° - 155.05 minutes

32.4° - 184 minutes

The results of this determination are in accord with the results obtained before. At 23.8° C., however, the generation time is greater than might be expected, as compared with that at 25.8° C. There is no reason either for doubting or believing this figure without further proof, which is impossible at this time.

From the various times found for one cell division it may still be said, as in other experiments, that 25° C. or thereabout is the optimum temperature for growth, and that there is a secondary optimum at 30° with a slight falling off at 28°.

There is still the bimodal growth, which was apparent when growth determinations were made by other methods.

Discussion:- In carrying out this experiment to determine the generation time certain details occured which have given rise to possible sources of error.

In the first place, in making the bacterial determination at the beginning of the experiment, dilutions too large for great accuracy were used, In the second plating, at the end of incubation, dilutions far too small were made, so that great colony development took place and actual count was impossible. Hence, estimation as accurate as is possible had to be resorted to.

While these errors may seem great from an absolute, quantitative viewpoint, they are, no doubt, comparative.For this reason the results mean as much in this case as though they were absolutely accurate quantitatively.

General Discussion

The methods used in this experimental work were of all grades, beginning with the gross growth determination which depends upon human decision for the recording of results, and ending with the determination of generation time, when the human element is eliminated and purely mechanical means substituted. It is interesting to note, however, the astonishingly close correlation of results in the two cases.

The use of the comparative method with barium sulphate allows great opportunity for error. In the first place, a small difference in amount of inoculum may determine, at least thru a short period of time, the temperature however incorrect it may be, for optimum development. This type of experiment necessitates a long period of incubation, which makes evaporation of the medium a factor because it takes place most rapidly at the

higher temperatures. Contamination during long periods of incubation is not improbable and might be a factor.

For these and other reasons, temperature regulation, etc., as means of determination is not satisfactory, although it pointed directly to the bimodal growth of B. phytophthorus in Uschinsky's synthetic medium.

Results obtained by plating methods at different periods are more accurate than those determined by standard comparisons, yet there is room for great experimental error.

Furthermore, accurate control of temperature thru long periods is difficult. There is also great opportunity for contamination, and here, more than before, a slight difference in the amount of inoculum shows and may create an optimum, not due to a greater growth but to a heavier inoculation. Here again bi-modalism was found, with the optimum at 25° C. and a sub-optimum at 30° C., as in the first case.

The last method used, namely, the determination of generation time, affords the most accurate and satisfactory means of obtaining the rate of bacterial growth. In this way the objections to the other methods are eliminated and chances for error reduced to a minimum. The time of incubation may be reduced to a minimum, the amount of inoculum determined quantitatively, and every detail satisfactorily worked out if careful manipulation is employed.

In a brief summarizing sentence, then, it may be said that even though some of the methods of growth determination employed seem crude all of them point to the same type of growth, developing under the influence of differences in temperature.

FIGURE 17 - GRAPH SHOWING GENERATION TIME OF B. PHTOPHTHORUS AT VARIOUS TEMPERATURES

FIGURE 18 - GRAPH SHOWING RECIPORCAL OF GROWTH DETERMINED BY GENERATION TIME.

FIGURE 17.

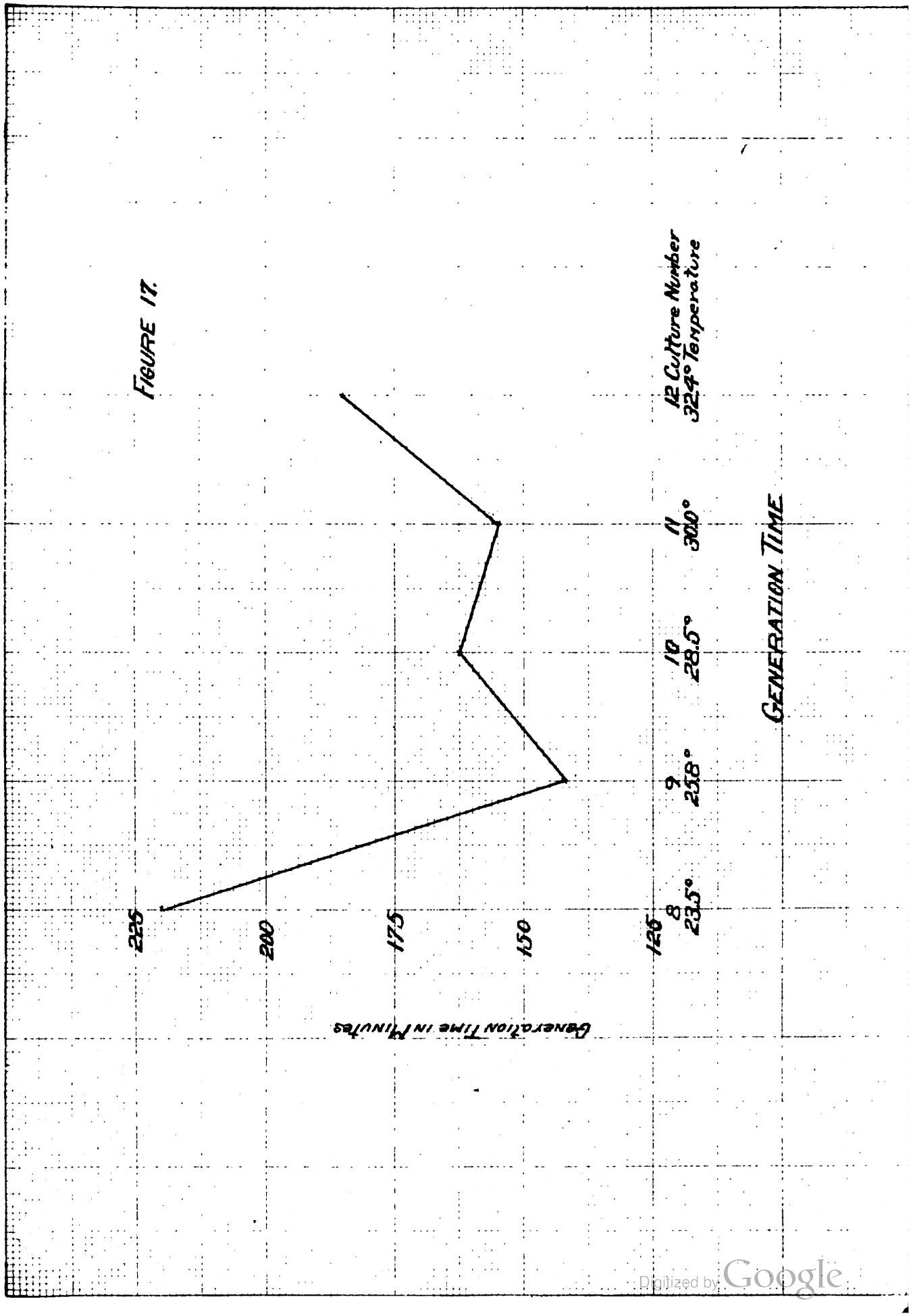
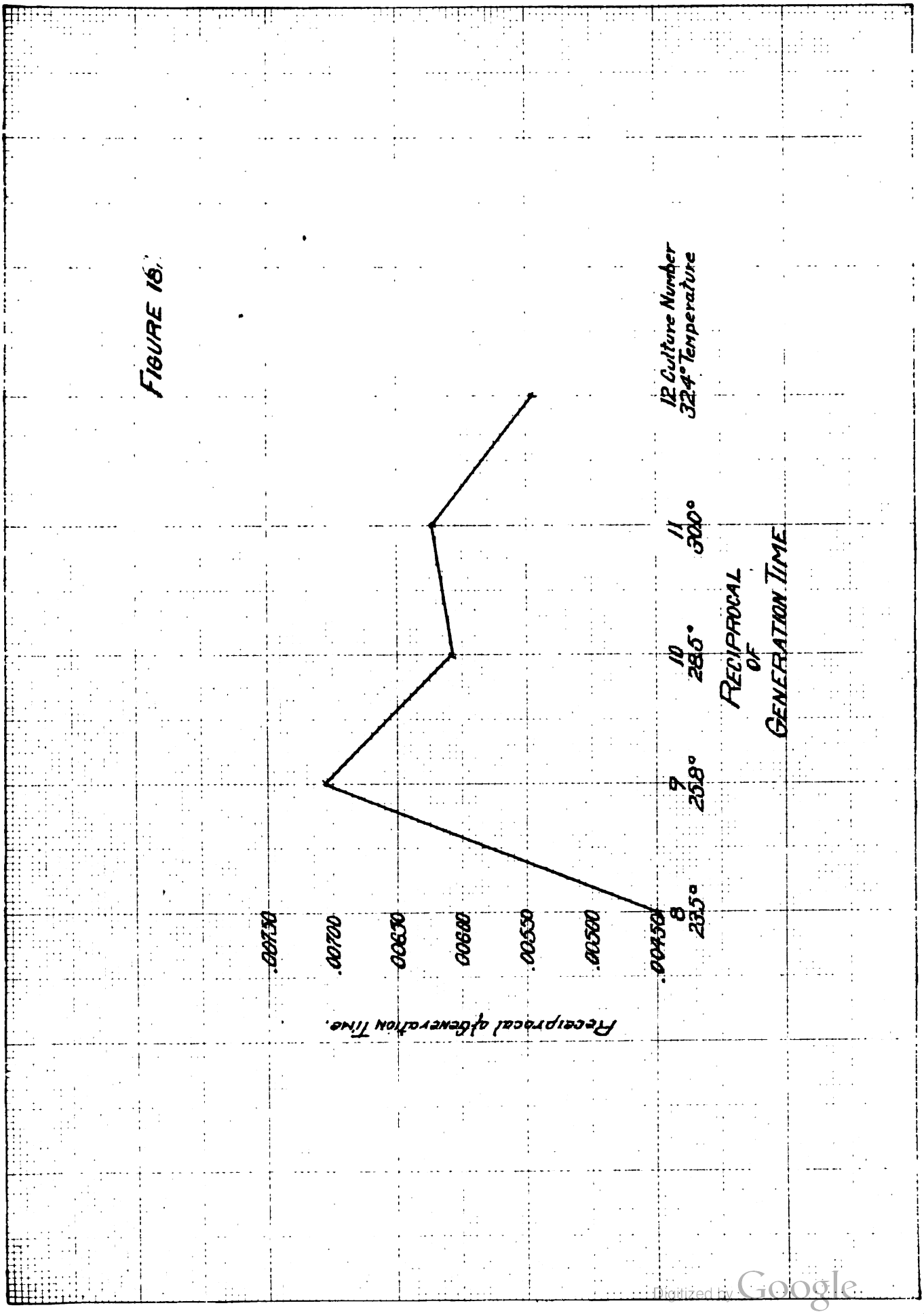


FIGURE 18.



General Summary of Temperature Experiments

(1) The optimum temperature for growth of B. Phytophthorus in Uschinsky's synthetic medium for a shor period is between 23°and 28° C., or at approximately 25°C., as demonstrated by comparison of growth with standard suspension of barium sulphate.

(2) When grown for periods longer than one week the growth in Uschinsky's medium points to 25°C as an optimum temperature, with 20° as a sub-optimum.

(3) Plates made from uniformly inoculated tubes at two days point to 25° C. as the optimum temperature for growth, with a sub-optimum at 30° and a falling off at 28° C.

(4) The length of generation time shows 25°C. to be the optimum temperature for growth, with a sub-optimum at 30° and a falling off in growth at about 28°C.

(5) It is, therefore quite firmly established that Bacillus phytophthorus shows a bimodal growth in Uschinsky's synthetic culture medium.

THE INFLUENCE OF MOISTURE ON THE DEVELOPMENT OF BLACKLEG

All workers dealing with the environmental factors concerning blackleg agree that the two principal factors are moisture and temperature. Which is the more important no one can tell, for low temperature necessarily follows a high moisture content of the soil. It has been noticed by Morse (8), Jones (5), and others that for a severe attack of blackleg a low temperature with considerable precipitation at the beginning of the season is necessary. If the ground is low it would naturally be moist and the evaporation of the moisture would give a low temperature and thus give, in a normally dry season, conditions which are favorable to the development of the disease.

So far as can be found in the literature available, no experimental work has been carried on demonstrating the effect of moisture on the development of the disease, with a constant temperature. Because of this it was decided to grow the potato plants at varying moistures at a constant temperature to see whether difference in the water content of the soil had to do with the severity of the disease.

First Series

Technique : A light sandy loam was used in order to approximate a good potato soil. The moisture holding capacity was determined on a saturated soil basis and found to be 30%. With this as a working hypothesis cans were filled with the soil and tubers of the Irish Cobbler variety planted. Immediately after planting the moisture content was so manipulated so as to

have three cans at 20% moisture, four cans at 25% moisture, and three cans at the water holding capacity, or 30%. The moisture content varied slightly at the extremes, the cans at 30% probably were actually at 28%, and those at 20% at about 21%. The temperature at which the plants were grown was 72° F. or 22° C. Before they were planted the tubers were very carefully selected and placed in 1:1000 corrosive sublimate to make them as free from disease as possible, and then rinsed in sterile distilled water to remove the last traces of the chemical.

The potatoes were allowed to grow until they were three or four inches high at the optimum moisture for their development. It was found that the plants at 25% moisture were up within two weeks, that the ones at 30% moisture came up about three days later, and that more than a week later the ones at the low moisture came thru the ground. Due to this irregularity in coming up when they were all inoculated the plants of the larger growths were considerably higher than the ones that came up later.

When the plants had grown to the above mentioned height they were inocculated in the following manner with a young agar slant culture of B. phytophthorus. The soil was removed from the tuber and a space about 5 m.m. square was pricked considerably with a flamed needle. In this pricked portion some of the culture was placed and then forced into the tuber tissue. The soil was then replaced and the moisture kept at the desired concentration.

In a very short time the plants at the high moistures, checks and inoculated ones alike, were much higher and more

spindling than those at either of the low temperatures. The plants were allowed to develop for four weeks, it being thought that in that time the disease should show itself in some way on the above ground parts. Since there were no such effects at that time, the plants were removed from the cans and examined.

Results:- It was found that all of the inoculated tubers showed degrees of rot. The ones at the 30% moisture had entirely rotted and all but the skin had nearly disappeared. At 25% moisture the tuber was entirely rotted but had not so nearly disappeared as in the former case. At 20% little rot had taken place in the tubers inoculated. There was a rather limited decayed area around the point of inoculation.

The plants showed varying effects of the disease underground. The ones at 30% showed numerous lesions all over the underground stems and roots that were close to the tuber itself. There was a noticable lack of development of the root system. On the plants at 25% there was no sign of any lesions at any considerable distance from the stems and roots touching the tuber. On the plants at 20% moisture there were no lesions to be found on any of the stems or roots near or remote from the tuber.

Summary:- From the data obtained from this experiment little may be told in a definite way as the exact effect of moisture on the development of the blackleg disease of the potato. It does point to some rather interesting facts concerning the relationship of moisture to the normal development of the potato plant. The relative speed of germination of the seed has been

referred to, also the relative height of the plants.

The strength and color of the plants was fully as striking as the size. The plants that were grown at the 20% moisture were short and stocky. They were of an extremely dark green color, giving the appearance of a perfectly healthy plant. Had it not been for the possible comparison with the larger plants, no abnormality could have been discerned by the average eye.

The plants at 25% moisture were healthy, vigorous, and dark green, the stems were stout, and the whole effect of the growth was one of normality.

The plants grown at 30% moisture were the ones which would be picked out, even though no normal ones were near, as being abnormal. They were tall and spindling and were yellow, as are all plants which are grown where there is too much moisture. The leaves were thin and the whole general impression produced was one of weakness and abnormality. For a comparison of the types of growth see figure 19.

Conclusion:- Because the results of the inoculations were so little noticable it was decided to try another series in which the tubers were to be inoculated shortly after they had been planted In this way it was thought that the plants could be made to show the effect of the disease above ground.

Figure 19 - Effect of varying moistures on growth of potato plant. A 1 = 20% , A 2 = 25% A 3 = 29%.

Second Series

Technique:- A medium sandy soil was used in this series, the water holding capacity being 31.5%, based on a saturated soil. The series consisted of fifteen cans, each containing approximately 12 kilos of the soil at its normal moisture content.

The potatoes which were used for seed were carefully selected, certified, Early Ohio's. They were allowed to stand in 1:1000 corrosive sublimate for 5 minutes to remove any spores or organisms on the surface. After this treatment they were thoroughly rinsed with sterile distilled water to remove the corrosive sublimate adhering to them. The tubers were then planted and the water content of the soil adjusted to the desired percentage. The series consisted of three cans at each of the following moistures: 18%, 22%, 26%, 29%, and 31%. The temperature at which cans were kept was approximately 20°C.

After the tubers had remained in the soil four days, one can at each of the moistures was inoculated with a vigorous, young, agar culture of Bacillus phytophthorus. The inoculation was accomplished as follows: a portion of the tuber was washed thoroughly with sterile distilled water and then a small area pricked with a sterile steel needle. In this punctured area was placed some of the culture mentioned above. The soil was then replaced and the plant allowed to develop. The check plants were treated in the same way, with the omission of the causal organism.

The plants came thru the ground in much the same order found in the first series. The plants came thru the surface of the soil first in the can with 26% moisture. Then came the plants in cans containing 22% and 29% moisture, in the order named. At the extremes, 18% and 31%, the plants were much slower in appearing above the surface or else did not appear at all.

As in the first series, the plants at the higher moistures were tall, yellow, and spindling, with a general unhealthy appearance At 26% moisture they looked most nearly normal.

At the end of six weeks the plants were removed from the cans and examined.

Results- 18% moisture, uninoculated:- No plants came thru the ground in either of the cans. The tubers were as firm as when planted and showed no signs of rot. There were sprouts about 5 cm. long on the tubers in each can. There was also a fairly good root development.

Inoculated:- No plants showed above the surface of the soil, but there were sprouts as in the uninoculated cans. There was considerable discoloration around the point of inoculation, but no rot could be distinguished.

22% Moisture Uninoculated:- The plants were 25 cm. high, healthy, dark green and vigorous. The tubers were perfectly sound, showing no signs of any rot.

Inoculated:- The plants were 25 cm. high, with a good color and a vigorous appearance. Around the point of inoculation there

of inoculation there was considerable discoloration, showing a trace of soft rot. The remainder of the tuber was perfectly sound.

26% Moisture Uninoculated:- One of the check plants was 45 cm. high, with a good healthy appearance. The other plant was but 25 cm. high, and showed low vitality, with the typical yellow, weak appearance. In neither case did the tubers show any signs of rot.

Inoculated:- The plant was 45 cm. high with a good, vigorous, green growth. In the tuber there was considerable rot around the point of inoculation. In this instance there was not as much discoloration as in the first two cases.

28% Moisture Uninoculated:- Check plant No. 1 did not break thru the ground but had a considerable sprout development. The tuber itself was partially rotted on one side, the rest being normally sound. Check plant No. 2 was 40 cm. high, yellow, and spindling, exhibiting all of the characters of plants grown in excessive moisture. The tuber was entirely sound.

Inoculated:- There was no plant above the surface of the soil, and the tuber was entirely rotted, leaving no trace of sprouts if there were any.

31% Moisture Uninoculated:- Check No. 1 was 25 cm. high and spindling. The tuber was perfectly sound. Check plant No. 2 was but slightly above the surface of the soil, and was weak and yellow. The tuber was partially rotted.

Inoculated:- In this can there was no plant growth above the surface of the soil, and no sprouts were visible. The tuber was entirely rotted.

Discussion:- The inoculated tubers kept at 18% moisture showed no signs of any rot. There seemed to be more discoloration in the inoculated tuber than in the checks, yet there was no indication of any rot at all. This might be due to the fact that the blackened portion was enlarged because of a slight growth of the bacteria in the immediate area. At any rate, even if growth of the organism did take place, very little progress was made.

At 22% moisture there was the usual discoloration of the points of inoculation both in the checks and the inoculated plants. In this case, however, there was a slight, though not very definite indication of rotting. The darkened area was much larger in the inoculated tuber than in the others, which might lead one to believe that the bacterial development had some influence on the amount of area darkened.

At 26% there was a distinct rotting of the tuber, not extensive, but of such a character as to be certainly a soft rot. The check tubers showed only the typical black streaks at the points of inoculation.

At 26% and 31% there was complete rotting of the inoculated tubers. Some of the check plants exhibited considerable rot at these moistures, which is significant, but in inoculated plants it was perfectly obvious that rot took place more quickly and completely than in the check plants. In the inoculated tubers there was no sign of any plant or sprout development, while in the check plants in nearly every case there was a top showing above the surface of the soil.

In no case did the stems or roots of the underground portion of the plant show the black streaks so typical of this disease. No doubt this was due to the rather short time during which the experiment was continued.

Summary:- The experiment under discussion seems to show that the tuber rot of potato tubers is more severe at the soil moistures and that it is apparently checked by low soil moisture. It may be summarized as follows: the development of the blackleg disease of the potato plant and tuber seems to be directly proportional to the amount of moisture in the soil, providing this moisture is not too abundant for the development of the plant.

GENERAL SUMMARY

(1) The optimum moisture content of the soil for the optimum development of a so-called "normal" potato plant in a sandy loam soil is about 25%, the water holding capacity is about 30%.

(2) In sandy loam soil the potato plant develops poorly at or below 20% moisture, and also at the moisture holding capacity.

(3) The development of the disease seems to be directly proportional to the amount of moisture in the soil, within the growing limits of the plant.

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